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DECAY IN MERCHANTABLE BLACK CHERRY ON THE ALLEGHENY NATIONAL FOREST[1]

ROSS W. DAVIDSON[2] AND W. A. CAMPBELL[3]

(Accepted for publication May 7, 1943)

INTRODUCTION

The botanical range of the black cherry (*Prunus serotina* Ehrhart) includes most of the eastern half of the United States and adjacent areas of eastern Canada. It is commercially important as a timber tree from West Virginia to western New York. Black cherry is one of the most important tree species in the unburned, second-growth, northern hardwood stands of the Allegheny Plateau in Pennsylvania, and, according to Ostrom (15), becomes more important as the number of short cutting cycles increases. Downs (11) has shown that in the mixed hardwood stands of northwestern Pennsylvania black cherry is abundant in the second growth, and both seedlings and sprouts gain early dominance over the slower growing tolerant species. He states that such stands generally become two-storied, the black cherry forming a large-crowned overstory, while the suppressed and intermediate sugar maple, yellow birch, and beech occupy subordinate positions and are of smaller size for the same age. Based on cubic-foot volume these second-growth stands were composed of black cherry 34.2 per cent, sugar maple 19.8, beech 14.6, yellow birch 8.1, and red maple 6.9 per cent. The 1936 glaze storm reduced black cherry to 20.6 per cent of the total volume, second to sugar maple, which was raised to 26.5 per cent. Black cherry probably will not produce lumber of such good quality as the slower growing sugar maple because its dominant position in the stand results in more limby trunks and greater damage from glaze.

Black cherry was early considered valuable for furniture and engraver's blocks; and most of the old-growth stands have been cut. Black cherry lumber now comes largely from second-growth stands and good quality material is difficult to obtain. Its suitability as pulp wood has increased its demand by paper mills operating in the Allegheny Region. This species also is used for chemical wood, but is not considered so desirable as are beech and maple. When this study was made, stumpage value of black cherry was intermediate between that of beech and sugar maple. With the rapid increase in volume in the second-growth stands now coming into timber production, its value should increase.

[1] In cooperation with the Allegheny Forest Experiment Station and the Allegheny National Forest. Special thanks are due A. F. Hough, J. C. McClellan, and J. B. Smith for their assistance.

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(Photo by A. F. Hough.)

FIG. 1. Nearly pure 116-year-old black cherry on Camp Run, near Westline, Pennsylvania. These trees were from 10 to 26 inches d.b.h, and had an average merchantable length of 51 feet. Many of the trees had small crowns, which were bady damaged by the 1936 glaze storm. Very little reproduction had established itself under the denser portions of the stand, but the larger openings had seeded in almost solidly to sugar maple.

The present paper gives the results of a study of decay in merchantable black cherry. This information should be of value in managing these second-growth stands for high quality timber production.

SOURCES OF MATERIALS AND METHODS

Areas Studied[4]

The investigators had available for the intensive part of this study a 20-acre sale area of dense, almost pure old-growth black cherry, 116 years old at Camp Run, near Westline, Pennsylvania (Fig. 1). The trees there were tall and straight and ranged from 10 to 26 inches d.b.h. and 100 to 115 feet in total height, with an average merchantable length of 51 feet (Table 1).

TABLE 1.—*Comparative information on merchantable black cherry trees in three logging areas on the Allegheny National Forest*

Area	Basis	Average age	Average stump diameter	Average merchantable length[a]	Total volume	Cull			
						Butt rot	Trunk rot	Total rot	Per cent of rot
	Trees	*Years*	*Inches*	*Feet*	*Bd.ft.*	*Bd.ft.*	*Bd.ft.*	*Bd.ft.*	*Bd.ft.*
Four Corners	21	52	18.5	33	3,980	90	0	90	2.3
Libby Run	20	120	25.7	46	13,520	75	750	825	6.1
Camp Run	281	116	20.1	51	101,910	2,615	8,935	11,550	11.3

[a] Includes only logs cut and milled and logs that would have been milled had they not been culled for decay. Portions of trunks left in the woods because of bad checks or bad form were not included in merchantable length or board-foot volume.

On Libby Run, also near Westline, Pa., a small sample was taken from a 120-year-old stand of more open-grown cherry. The trees on this area were mixed with other hardwoods and were larger in diameter than those at Camp Run. They had large branching tops and were from 16 to 28 inches in diameter, with an average merchantable length of 46 feet.

At Four Corners, 12 miles southeast of Kane, Pennsylvania, a third sale area, which consisted of scattered old-growth maple and beech interspersed with groups of 52-year-old black cherry and other hardwoods also was sampled. The merchantable cherry, which was open-grown with large branching tops, was from 11 to 20 inches in diameter with an average merchantable length of 33 feet.

[4] Mr. A. F. Hough has kindly supplied additional data on these stands. He states that the Libby Run cherry originated from a blowdown of virgin beech-maple forest 120 years prior to 1940 and Camp Run after a blowdown of virgin beech-maple forest 116 years prior to 1940. The Four Corners cherry originated after a heavy cutting of hemlock-beech forest and the trees became limby and forked due to lack of crowding by trees of similar growth rate.

The Libby Run area had been subjected to logging in 1894. From the Camp Run stand 4 M.B.F. in sawlogs of various sizes was selectively cut during the period 1914 to 1940. As computed on sample plots 21 M.B.F. per acre was left, which grew at the annual rate of 485 board feet per acre. At the time of the 1940 cutting, a total volume of 34 M.B.F. per acre had been produced by the residual trees, disregarding all natural mortality.

In addition to the study of decay in these 3 merchantable stands a general search of many areas in the Allegheny National Forest was made for cherry heartwood-decaying fungi, particularly for those producing conks on living or dead trees. Whenever possible, cultures were obtained from these conks for comparison with cultures from heartwood decay.

Method of Collecting Data

Information on individual trees was obtained by following the logging crews on the areas sampled. Stump and top diameters and length of each log were recorded for all sound and decayed trees. The diameter of decay on the ends of the logs was recorded and the extent of decay was measured or estimated. Isolations of the causal fungi were made from decay samples, and these were identified by comparison with isolates from identified conks.[5]

Method of Scaling

The Scribner Decimal C log rule, standard with the Forest Service on the Allegheny National Forest, was used in scaling all logs. In determining volume, only the merchantable portion of the trunk or what would have been, except for decay, was included as total merchantable volume. Parts of trunks left in the woods because of butt check or bad form were not included. No attempt, however, was made to subtract cull for check, sweep, and defects other than rot from logs taken to the mill.

DECAY IN RELATION TO TYPE OF STAND

Some stands contain much decay, whereas others contain very little; a difference often difficult to explain. Usually, however, decay reflects age, severe fire damage, or other factors. The difference in the amount of cull from decay on the 3 areas studied (Table 1) can be explained in part by the difference in age. The black cherry at Four Corners was only 52 years old, or less than half the age of the stands at Libby Run and Camp Run. As would be expected, cull from decay was only 2.3 per cent, as compared to 6.1 and 11.3 per cent, respectively, in the two older stands. Also, the decay that caused cull in the Four Corners trees was mostly that of the butts, whereas in the others it was chiefly in trunks or tops.

In comparison with the Camp Run and Libby Run stands, the Four Corners' black cherry contained more butt rot than was expected in such young trees. The data (Table 1) do not account for this difference, and no reason for it was found in a more detailed study of the area. The 21-tree sample possibly did not suffice for accurate information, but a thorough survey of all cherry stumps over much of the sale area indicated that butt infections were as frequent as or probably more so than in the Camp Run stand. Theoretically, this may be explained by assuming that trees with butt decay did not survive for 116 years. Actually, however, very few 116-year-old trees were weakened sufficiently by butt rot to wind throw.

[5] The identifications of the fungi found fruiting on black cherry were made or verified by L. O. Overholts of Pennsylvania State College.

TABLE 2.—*Summary of cull caused by different fungi in 281 trees on the Camp Run Sale Area*

Fungi	Distribution of cull		Infections upon which cull is based
	Per cent	*Board feet*	*Number*
Poria prunicola	36.6	4,225	50
Poria mutans	13.4	1,545	14
Fomes pinicola	9.0	1,040	21
Polyporus sulphureus	6.6	760	18
Polyporus spraguei	2.7	310	10
Trametes serialis	1.5	175	3
Poria sericeo-mollis	1.3	150	2
Polyporus berkeleyi	0.9	100	5
Poria inflata	0.8	95	5
Polyporus schweinitzii	0.6	70	2
Miscellaneous fungi	3.1	360	3
Undetermined fungi			
Brown rot	3.4	390	12
White rots	18.2	2,100	46
Mixed rots	2.0	230	8
Totals	.....	11,550	199

Of the two older stands, the one at Camp Run was studied more carefully and yielded most of the information on types of decay, species of rot-inducing fungi (Table 2), and their extent and mode of entrance. From the standpoint of comparison with the Four Corners area, the Libby Run sample is more valuable, because these trees were open-grown and could be pictured as the Four Corners trees 58 years later. In spite of numerous overgrown branch stubs in the Four Corners trees, there were few infections by top-inhabiting fungi. A few *Fomes pinicola* (Sw.) Cke. conks were found by searching the entire area (Table 3) but no infections of *Poria prunicola* (Murr.) Sacc. and Trott., the principal cull-producing fungus in older stands, were found.

The Camp Run trees formed crowded stands. Most of them were growing very slowly and bore numerous dead branch stubs, a condition that had existed for years and had resulted in early infection by top-rotting fungi and a consequent high proportion of cull. The Libby Run trees bore large tops with numerous big dead branches and branch stubs fairly low down on the trunks. These contained numerous top infections, but only a small number resulted in cull.

THE DECAYS OF BLACK CHERRY

Points of Entrance

Decays may be divided into two groups depending upon their location in the tree. Those in the base are referred to as butt rots; those in the trunks as trunk rots. The stands studied on the Allegheny Plateau had not been injured by fires, and butt rots were mainly associated with logging wounds, dead roots, drouth injuries, or obscure basal lesions. Most of the

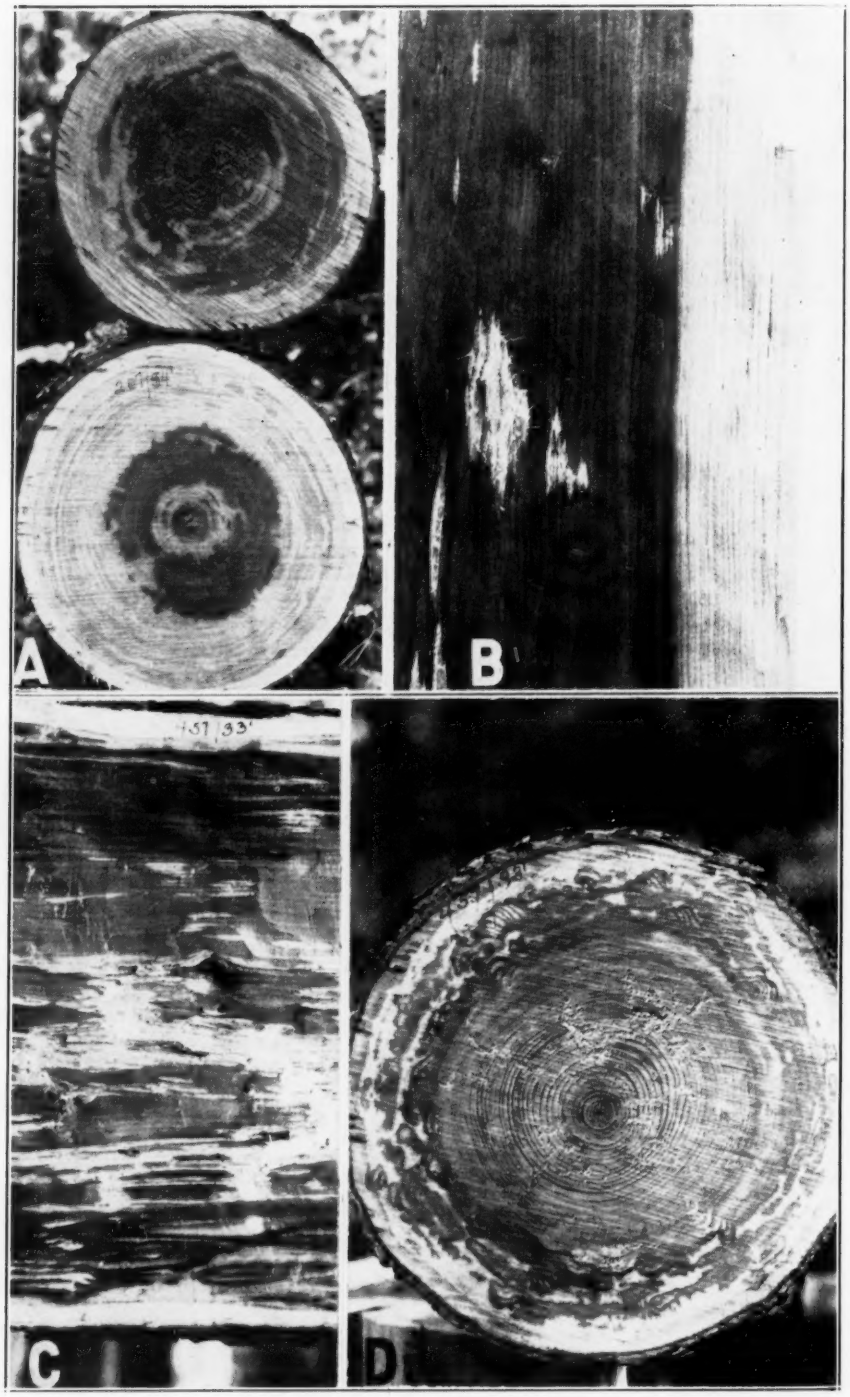

FIG. 2. *Poria prunicola* rot of black cherry. A. Red discoloration of the heartwood as seen on the end surfaces of recently cut logs. The cut end of the lower log was at 54 feet, the top log, corresponding to the opposite or top end of the same log, was at 66

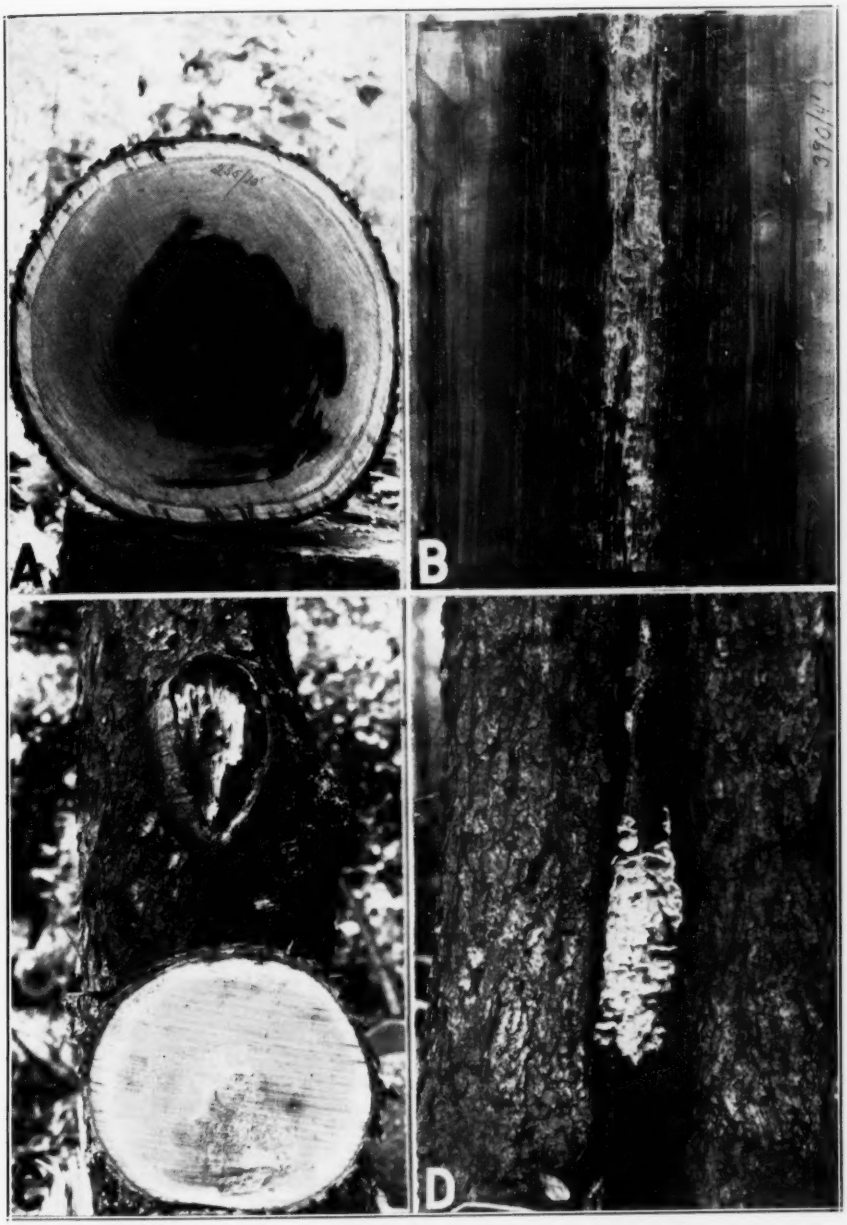

FIG. 3. A. and B. *Poria mutans* rot in black cherry. A. End cut showing the water-soaked nature of the rot. B. The pocket or piped advanced stage of the rot. C. A rot-infected branch stub approximately 4 inches in diameter. The brown rot, which had extended more than a foot below the stub, was caused by *Poria inflata*. D. Conks of *Polyporus subcartilagineus* on a butt wound. This fungus causes a brown rot.

feet. The rot infection originated above 66 feet in the tree and was working down the trunk. B. White pockets of advanced decay in the red-stained heartwood. C. A thoroughly rotted log split open to show the white pockets and streaks of advanced decay. These white pockets are not conspicuous on end cuts, even in badly rotted logs. D. Advanced decay in a thoroughly rotted top showing how the decay may encroach upon the sapwood.

TABLE 3.—*Summary of all fungi isolated from butt and trunk rot in black cherry*

Fungi	Decay infections from all stands			Decay infections from Four Corners and other young stands			Decay infections from Camp Run and Libby Run		
	Total	Butt	Trunk	Total	Butt	Trunk	Total	Butt	Trunk
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Coniophora cerebella Pers.	8	8	0	7	7	0	1	1	0
Corticium lividum Pers. ex Fr.	1	0	1	0	0	0	1	0	1
Fomes pinicola (Sw.) Cke.	32	2	30	6	1	5	26	1	25
Hydnum sp.	2	0	2	0	0	0	2	0	2
Omphalia campanella Fr.	2	0	2	0	0	0	2	0	2
Polyporus balsameus Pk.	2	0	2	2	0	2	0	0	0
" *berkeleyi* Fr.	9	9	0	3	3	0	6	6	0
" *fibrillosus* Karst.	1	1	0	0	0	0	1	1	0
" *frondosus* Dicks. ex Fr.	2	2	0	1	1	0	1	1	0
" *schweinitzii* Fr.	2	2	0	0	0	0	2	2	0
" *spraguei* Berk. and Curt.	14	14	0	0	0	0	14	14	0
" *subcartilagineus* Overh.	3	2	1	2	2	0	1	0	1
" *sulphureus* Bull. ex Fr.	28	5	23	1	0	1	27	5	22
" *versicolor* L. ex Fr.	1	0	1	1	0	1	0	0	0
Poria cocos (Schw.) Wolf	3	3	0	2	2	0	1	1	0
" *inflata* Overh.	5	0	5	0	0	0	5	0	5
" *mutans* Pk.	18	7	11	2	2	0	16	5	11
" *prunicola* (Murr.) Sacc. and Trott.	62	0	62	0	0	0	62	0	62
" *sericeo-mollis* (Rom.) Baxter	10	6	4	6	4	2	4	2	2
" *xantha* (Fr. ex Lind) Cke.	1	1	0	0	0	0	1	1	0
Stereum rameale Schw. ex Burt	1	1	0	0	0	0	1	1	0
Trametes serialis Fr.	5	3	2	2	0	2	3	3	0
Totals	212	66	146	35	22	13	177	44	133

trunk rots were either associated with elongated trunk wounds or with dead-branch stubs larger than 3 inches in diameter.

Types of Rot

On the basis of color and texture, wood decays are classified as brown, carbonizing rots, or white, noncarbonizing rots. About two-thirds of the cherry rots are of the former type, and include those caused by *Fomes pinicola, Polyporus spraguei, P. schweinitzii, P. sulphureus, Poria cocos, P. sericeo-mollis* and *Trametes serialis.* The brown rots are very similar to each other in texture and color, and the fungi causing them usually can be identified only in pure culture.

The important noncarbonizing rots were caused by *Poria prunicola, P. mutans* and *Polyporus berkeleyi,* and can be identified usually on the basis of the following characteristics:

White-flecked Red Rot (*Poria prunicola*). Characterized by red discoloration of heartwood, extending sometimes throughout 20 to 40 feet of trunk. Ends of infected logs purplish-red covering most of end section or confined to smaller areas near center (Fig. 2, A). The red-colored wood is altered mechanically in definite pockets or areas of soft, white, flaky decay (Fig. 2, B). These pockets, few or abundant, are not conspicuous on end cuts, but plainly visible when an infected log is split (Fig. 2, B and C). Decay confined to heartwood except in old infections where it may encroach on sapwood (Fig. 2, D).

White-streaked or Piped Rot (*Poria mutans*). White, water-soaked, pocket or piped rot of heartwood (Fig. 3, A and B); may extend from injuries, up and down the trunk. The incipient stage generally dark and water-soaked (Fig. 3, A).

Light-brown Flaky Rot (*Polyporus berkeleyi*). A light-brown or yellowish, soft, flaky, butt rot (Fig. 4, A), recognized readily by peculiar sweet odor. Fungus probably enters small dead roots; conks were found several times on soil near bases of infected trees (Fig. 4, B).

CONKS ON LIVING BLACK CHERRY

Conks of *Fomes pinicola,* more prevalent on living black cherry than those of other fungi (Fig. 5, A to C). Commonly found on the larger trees associated with branch stubs (Fig. 5, A and B), branch wounds, or trunk wounds exposing heartwood. They also may develop at base of tree from butt wounds or wounds left by death or severance of companion stems. Sporophores of other *Fomes* common to hardwoods were infrequent on black cherry.

Conks of *Poria prunicola,* common on dead fire cherry (*Prunus pennsylvanica* L. f.), throughout Allegheny National Forest. No conks found on living black cherry and the only one collected on that species was on a rotted log, approximately 12 inches in diameter, that had been in contact with the ground for some years.

Several species of rot fungi fruited infrequently over a period of years. In certain seasons *Polyporus sulphureus* was common on living trees and decaying logs and stumps. *Polyporus spraguei* fruited abundantly on

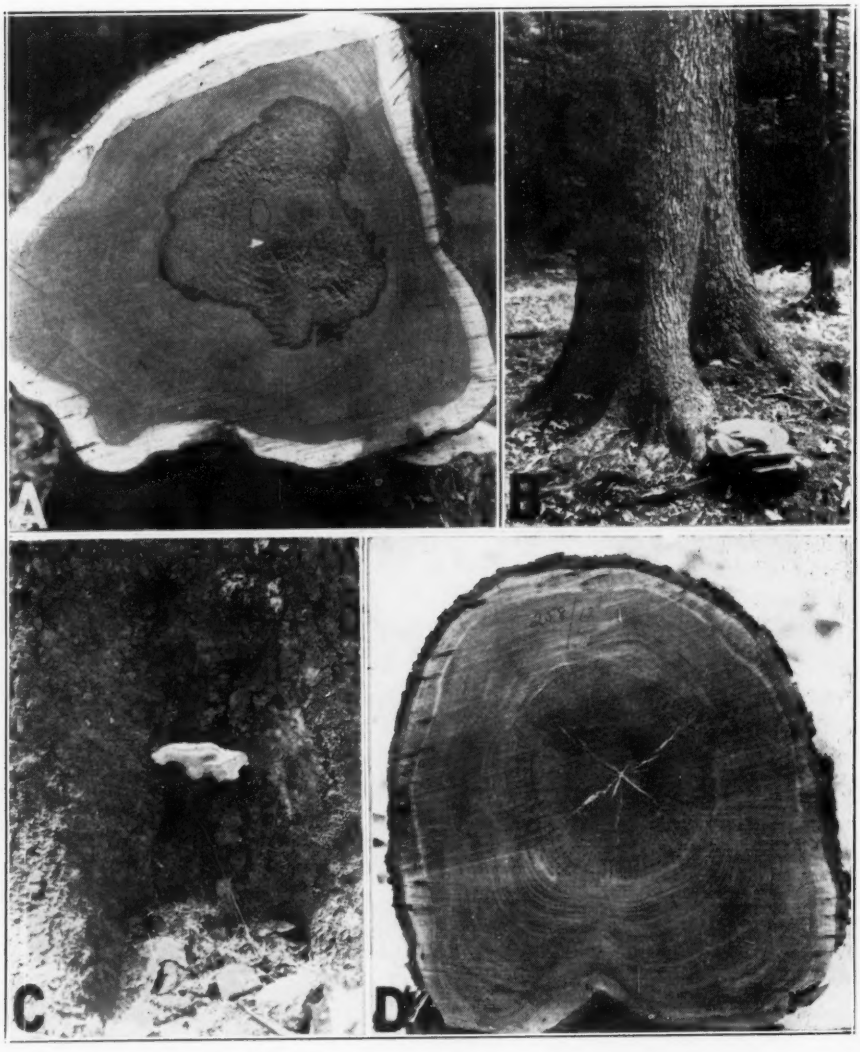

FIG. 4. A and B. *Polyporus berkeleyi* on black cherry. A. Butt rot as exposed by the stump cut. This rot was limited to the stump or extended but a short distance into the butt. B. A conk at the base of a large tree near Camp Run, photographed in August 1940. A conk was produced on the same spot in 1939. C and D. *Polyporus spraguei* on black cherry. C. A conk that developed from a small stump wound on a recently cut stump. This fungus did not fruit on living infected trees but was common at the base of dead snags or on the stumps of recently cut trees. D. Brown rot caused by the fungus.

recently cut stumps and cut surfaces of logs that exposed rot, but was not observed on living trees (Fig. 4, C). *Poria sericeo-mollis* fruited rather commonly on large wounds in living black cherry in 1936 and 1937. In

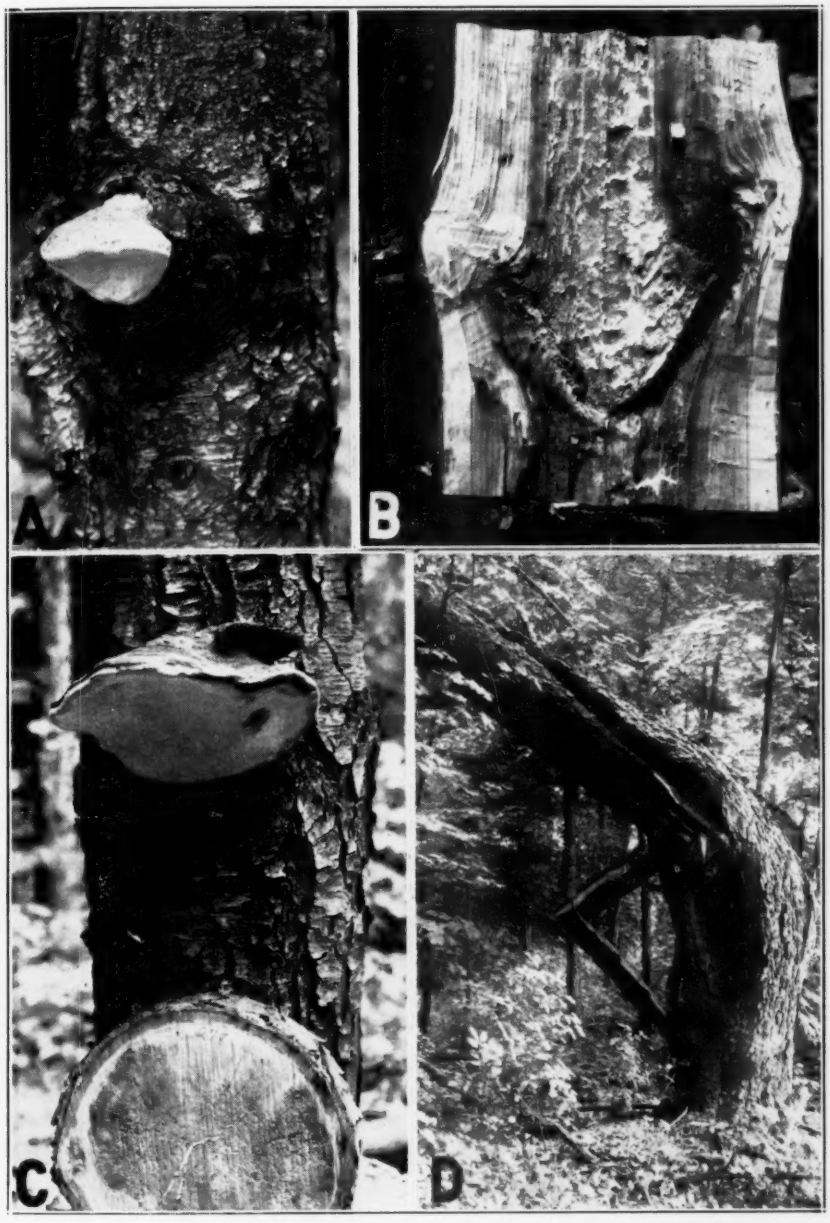

FIG. 5. A. to C. *Fomes pinicola* conks and rot of black cherry. A. Conk on a nearly healed 3-inch branch stub. B. An infected top split open to show the branch stub that served as the infection court and the badly rotted heartwood. C. A large conk associated with a branch stub on a 52-year-old tree. The diameter of the rot column one foot below the conk included practically the entire cross-sectional area of the heartwood. Badly rotted tops commonly break under strain caused by wind or glaze. D. A badly rotted black cherry approximately 24 inches d.b.h., which broke off in a windstorm.

1940, conks were not collected on living trees, but several were found on badly rotted logs. *Poria mutans* was observed fruiting abundantly on large trunk injuries in several instances. Most rot infections, however, were unaccompanied by fruiting. *Coniophora cerebella* was collected only on dead wood during a rainy period in the fall of 1940. A large fruiting body of *Polyporus berkeleyi* was collected from the base of a black cherry at Camp Run in 1939. A conk developed at the same spot again in 1940 (Fig. 4, B). One other fruiting body was found in the same area in 1940. *Polyporus schweinitzii* fruited in 1939 on one of the trees found to be infected with it at Camp Run. *Polyporus subcartilagineus* (Fig. 3, D) was found twice fruiting on decayed butt wounds, but the fungus was unimportant on the logging areas studied. *Polyporus balsameus* was collected once on the badly decayed surface of a large butt wound. *Trametes serialis* was collected once on rotten wood associated with a split fork.

The other heart-rotting fungi listed in table 3 were not observed to fruit on living cherry and were identified from cultures isolated from decay.

PREDICTING CULL FROM DEFECTS IN STANDING TREES

Very few trees reach merchantable size without injuries, which serve as infection courts for wood-rotting fungi. Small injuries usually heal without decay, but large trunk and butt wounds, and particularly wounds caused by large branch stubs, heal slowly and often become badly rot-infected. Trees reserved for a future cut, if they already possess large wounds or are injured in logging, often decrease rather than increase in merchantable volume after a period of years, because of advancing decay. A number of black cherry trees on the different areas had conspicuous trunk and butt wounds. In table 4 these are analyzed as to size in relation to age of stand and extent of rot resulting in cull. These data include all trees with larger trunk and butt wounds, since these were easily recognized.

Broken branches and dead stubs were very common in stands of all ages. Many of these breaks were of recent origin and dated from the glaze storm of 1936. Older breaks were especially numerous in the 116-year-old trees, and were associated with approximately half of the decay cull found in the stands. In many trees decayed by *Poria prunicola*, the branch stubs that served as entrance courts had healed over completely. Most of the stems could not be dissected to determine the size of the stub responsible for infection, but, for the small number examined critically, the stub diameter was found to be over 3 inches. Thirty-two dead infected stubs in the 116-year-old trees had an average diameter of 6.5 inches with an average rot extent of 15 feet. All of these stubs were "old," that is they were rotten or were cavities in the trunk from which the stub itself had disappeared. Only 2 rot-infected stubs under 3 inches in diameter were noticed and these did not result in cull. Decay from branch stubs in black cherry rarely causes serious cull for 10 to 15 years. No cull was caused by *Polyporus*

versicolor and *Stereum rameale*, 2 fungi that were found to cause limited rot infections in top breaks caused by glaze (4).

TABLE 4.—*Extent of rot and cull associated with butt and trunk wounds in black cherry*[a]

Stand age	Butt wounds				Trunk wounds				Split forks[b]		
	Number	Average length wound	Average extent rot	Average cull	Number	Average length wound	Average extent rot	Average cull	Number	Average extent rot	Average cull
Years		*Feet*	*Feet*	*Bd.ft.*		*Feet*	*Feet*	*Bd.ft.*		*Feet*	*Bd.ft.*
116	10	5.5	10.2	100	13	10.6	17.3	150	2	18.5	90
52	11	4.3	6.5	30	1	3.0	8.0	60	5	11.4	60

[a] Includes all open wounds and readily detected healed wounds.
[b] If a fork had broken completely off the wound was classified as a trunk wound.

Only one fungus, *Fomes pinicola*, formed conks regularly on black cherry, and this fungus was found fruiting on 16, or half, of the trees it had infected. Most of these conks were in the tops in connection with dead stubs. Twelve conks of *Fomes pinicola* in the 116-year-old trees were associated with an average rot extent of 13.5 feet, and 4 conks in the 52-year-old trees were associated with 10.8 feet of rot. Conks of *F. pinicola* on a dead stub did not indicate more decay than the average decay associated with 32 stubs analyzed for the 116-year-old trees.

Some suggestions for estimating cull in black cherry are given in table 5.

DECAYS IN BLACK CHERRY IN RELATION TO MANAGEMENT

One reason for studying decay in a particular tree species is to determine if cull from rot will affect management for saw-timber production. Another reason is to determine how intensive management may affect the incidence and amount of decay.

The present study indicates that decay will not be serious during the length of time necessary to produce a maximum yield of high quality black cherry saw timber. The amount of decay in the 116-year-old trees at Camp Run, although causing 11.3 per cent cull, was not excessive; and 116 years seems adequate for a satisfactory yield of good quality logs.

Several of the 120-year-old trees yielded approximately 1,000 board feet each; several on the Four Corners area yielded 300 board feet in 52 years. These faster-growing trees did not contain as high quality clear lumber as the slower growing ones, but many trees at Camp Run contained a fair amount of fine clear lumber. If the Camp Run stand had been thinned carefully during the last 60-year period the final stand would probably have contained the same volume in fewer trees and may have had less decay. This conclusion is based on the fact that the Libby Run trees, which grew under less crowded conditions, contained a smaller proportion of decay.

However, trunk injuries resulting from selective logging would probably increase the amount of decay.

Some might question the ability of black cherry to withstand crowded conditions necessary for good height growth during the early development of the stand, but the Camp Run stand contained many trees of small diameter which had survived years of crowding. Also much small-diameter 52-year-old cherry at Four Corners had attained an excellent height of clear stems. These trees will probably produce a greater volume of fine-quality lumber during the succeeding 50-year period than was obtained from the

TABLE 5.—*Summary of suggestions for estimating cull defect in merchantable black cherry from wounds or dead stubs 10 years old or older*[a]

Type of injury	Age of stand	Extent of rot in relation to wound size
Butt or trunk wounds	40 to 70 years	Average extent of rot 1.5 times length of visible injury Average diameter of rot column equal to width of wound at widest part
	70 to 116 years	Average extent of rot 2.0 times length of visible injury Average diameter of rot column twice width of wound at widest part
Branch stubs	40 to 70 years	Ordinarily rot from stubs not sufficient to cause appreciable cull
	70 to 116 years	Average rot extent 15 feet below rotten knot holes or badly decayed branch stubs over 3 inches in diameter. Greatest danger from rot comes from stubs over 5 inches in diameter
Conks of *Fomes pinicola*	All ages	Average rot extent approximately 12 feet; above and below conk. Usually the larger the conk the more rot

[a] Based on the observation that in general the older the trees, the longer they have been exposed to infection, and the older the wounds tend to be. Usually it takes from 10 to 20 years for black cherry wounds to become badly rotted.

faster-growing 52-year-old ones. This will depend upon how well cherry of this type responds to opening up of the stand, and how much damage will result from wind and glaze storms.

Butt rot was not important from the standpoint of cull in merchantable trees, even though a number of strictly butt-rot fungi were present. In only a few trees had butt rot developed an excessive amount of cull or had become sufficiently extensive to cause serious weakening (Fig. 5, D).

Stand-improvement operations obviously should attempt to remove forked trees and eliminate multiple sprout clumps or reduce them to single stems at an early age. Other outstanding evidences of poor-risk trees are large trunk wounds and sporophores of *Fomes pinicola*. Dead branch stubs do not indicate individual poor-risk trees, but a stand with many large dead branches will eventually have numerous decay infections.

From the standpoint of decay, black cherry should be an excellent tree to manage for lumber production. It has a durable heartwood and is

affected by relatively few strictly heartwood-inhabiting fungi. Black cherry also grows rapidly and most heartwood decayers do not have time to cause excessive cull before merchantable size is attained.

DESCRIPTION OF BLACK CHERRY DECAY FUNGI IN CULTURE

The most practical means of determining the fungus responsible for decay in a particular piece of infected wood is by pure-culture methods. These methods have been described in detail by Davidson, Campbell, and Vaughn (10). By means of the pure-culture method 22 fungi were found to cause decay in black cherry on the Allegheny Plateau (Table 3). Nine of these, namely, *Corticium lividum*, *Polyporus berkeleyi*, *P. frondosus*, *P. spraguei*, *P. sulphureus*, *P. versicolor*, *Poria cocos*, *P. inflata*, and *Stereum rameale*, occurred as heart rotters in oak, and their cultural characteristics have already been described in detail (10). These fungi are listed in the key but no further description will be given. *Polyporus schweinitzii*, a species common to softwoods, has been described culturally by Childs (8) and further description of its cultural characteristics does not seem necessary. Only a few isolates were obtained for *Omphalia campanella*, *Polyporus fibrillosus*, and *Hydnum* sp. and these were not considered adequate for a description of the species in culture.

Detailed descriptions of cultures are given for 9 fungi that caused more or less extensive decay and for which a sufficient number of isolates from black cherry or other hosts were available to give some idea of the range of variations to be expected of each fungus in culture. These fungi are: *Coniophora cerebella*, *Fomes pinicola*, *Polyporus balsameus*, *P. subcartilagineus*, *Poria mutans*, *P. prunicola*, *P. sericeo-mollis*, *P. xantha*, and *Trametes serialis*.

Key to Black Cherry Decay Fungi, When Grown on Malt Agar

The following key is based on a classification system developed for oak fungi (10) and includes 9 fungi common to both oak and black cherry and 9 isolated only from black cherry. The cultural characteristics of the fungi causing rot in oak can be directly compared with those from black cherry by combining the two keys.

The first letter refers to mat color in 14 days in diffused light at room temperature for mats grown on malt agar in Petri dishes. A, white at all ages; B, at first white becoming yellowish or brownish in 14 days; C, yellow; D, brown; E, pink or orange; F, any other color not fitting in well with the first 5 divisions.

The second letter refers to the oxidase reaction, which is obtained by growing the fungi to be tested on malt agar to which has been added 0.5 per cent gallic or tannic acid (9). Brown-rot fungi usually cause no reaction or discoloration of these media; white-rot fungi cause a "reaction" or browning of the media under the fungus mats. O, negative reactors or brown-rot fungi; P, positive reactors or white-rot fungi.

The third letter refers to growth rate as mat diameters in centimeters. F, rapid, over 9 cm. in 7 days; I, moderately rapid, over 9 cm. in 14 days but less than 9 cm. in 7 days; M, medium, 5–9 cm. in 11 days; S, slow, 2–5 cm. in 14 days; V, very slow, less than 2 cm. in 14 days.

The numbers refer to microscopic structure. 1, clamps; 2, chlamydospores; 3, conidia; 4, oidia; 5, basidia; 6, basidiospores; 7, setae; 8, vesicular cells; 9, abnormal branching of hyphae; 10, all hyphae staining with eosin or erythrosin; 11, nonstaining hyphae present; 14, hyphae encrusted with crystals; 16, special structures or reactions described in descriptions of cultures.

In the key only those characters that are actually present are listed; characters not

listed should be considered absent or not readily observed. In separating the fungi with the same key pattern reference must be made to the cultural descriptions.

A–O–I–1–2, *Poria xantha;* *A–O–I–1–2–11, *Polyporus spraguei;* A–O–I–1–2–11, *Fomes pinicola;* A–O–M–1–2–10, *Polyporus balsameus;* A–O–M–1–2–11, *Trametes serialis;* A–O–M–1–2–11, *Poria sericeo-mollis;* *A–O–M–1–2–11, *Polyporus spraguei;*

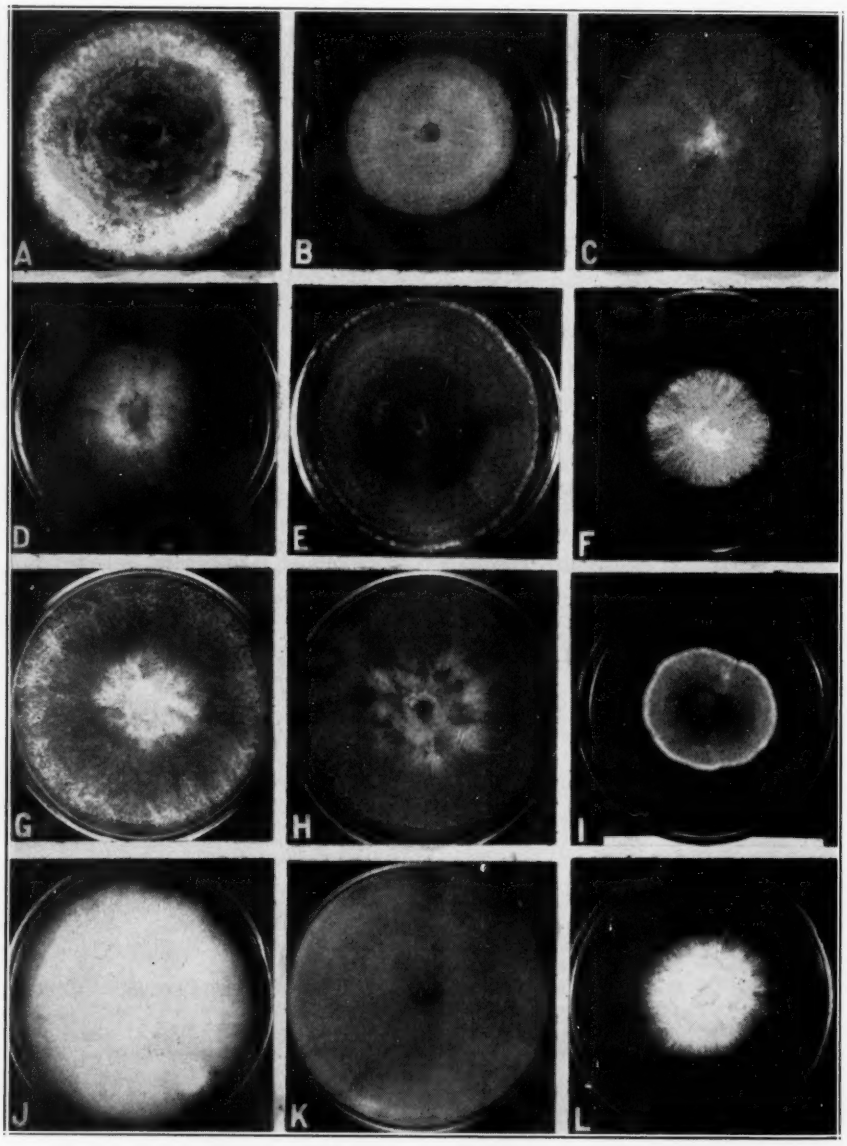

FIG. 6. Fourteen-day-old cultures of black cherry heartwood-inhabiting fungi. A, *Coniophora cerebella;* B and C, *Fomes pinicola;* D, *Polyporus balsameus;* E, *Polyporus schweinitzii;* F, *Polyporus subcartilagineus;* G and H, *Poria mutans;* I, *Poria prunicola;* J, *Poria sericeo-mollis;* K, *Poria xantha;* and L, *Trametes serialis.*

* Fungi whose cultural characteristics have been previously described in the oak decay study.

A–O–M–1–2–11, *Fomes pinicola;* *A–O–M–2–3–10, *Poria inflata;* *A–O–M–2–10, *Poria inflata;* A–O–S–1–2–10, *Polyporus subcartilagineus;* *A–P–F–1–11, *Polyporus versicolor;* *A–P–M–1–2–10–16, *Polyporus frondosus;* *A–P–M–1–2–10–16, *Hydnum erinaceus;* *A–P–M–2–5–6–11, *Polyporus berkeleyi;* B–O–F–1–11–16, *Coniophora cerebella;* B–O–I–1–2–11, *Poria xantha;* *B–P–F–1–11–16, *Stereum rameale;* *B–P–I–1–2–5–6–11–16, *Corticium lividum;* B–P–I–1–2–11, *Poria mutans;* C–P–M–11, *Poria prunicola;* *D–O–F–8–10, *Poria cocos;* D–P–M–11, *Poria prunicola;* *E–O–F–8–10, *Poria cocos;* *E–O–I–2–10, *Polyporus sulphureus;* E–O–M–1–2–10, *Polyporus balsameus;* E–O–M–1–2–11, *Poria sericeo-mollis;* *E–O–M–2–10, *Polyporus sulphureus.*

CONIOPHORA CEREBELLA PERS.

(Fig. 6, A, and Fig. 7, A)

Key Pattern.—B–O–F–1–11–16.

Growth Characteristics.—Growth rapid, mat filling dish in 6 to 8 days, at first fragile, radiating in loose cottony strands, white to "Light Buff" (18); in 14 days mat variable, either loose-cottony, azonate, evenly "Light Buff" to "Cream Color," or with "Sayal Brown" and "Avellaneous" appressed patches alternating with raised, cottony "Light Buff" to "Cream Color" areas; surface mycelium either appressed cottony or nodulose or tufted.

Hyphal Characteristics.—Hyphae that stain with eosin 2–8 μ (10) in diameter, some of the hyphae with multiple clamps arranged in whorls around the septa, these easily demonstrated in young cultures, more difficult to find in 14-day-old mats; cross-walls also present independent of clamps; staining content in older hyphae often arranged in threads of varying diameter leaving an irregular nonstaining wall; in 14-day-old cultures yellow or brown hyphae with irregularly spaced knobs and side branches are occasionally found in darker portions of the mat.

Type of Decay.—Brown butt rot. Eight isolations.

Remarks.—Fritz (12) isolated a fungus from balsam fir rot (Type A), which was characterized by clamp connections in whorls. Her description of this fungus in culture indicates that it is probably *Coniophora cerebella. Coniophora cerebella* has also been isolated from balsam fir rot in New England.[6]

[6] Isolated by R. W. Davidson from rot sent in by P. Spaulding and J. R. Hansbrough.

FOMES PINICOLA (SW. EX FR.) CKE.

(Fig. 6, B and C)

Key Pattern.—A–O–M–1–2–11 and A–O–I–1–2–11.

Growth Characteristics.—Isolates divided into two distinct growth forms (Fig. 6, B and C); the slow-growing strain in 14 days forming a mat 6–8 cm. in diameter, white, appressed, short-cottony or downy, zonate or azonate, occasionally somewhat pulverulent around the center; margin mostly white, even; fast-growing strain completely filling Petri dish, white or faintly white, azonate, appressed, fragile, thin matted-cottony.

Hyphal Characteristics.—Staining hyphae 2–5 μ in diameter, with many clamps; nonstaining fibrous hyphae 2–5 μ, smooth, no clamps; chlamydospores few or many, ellipsoid or very irregular in shape, 6–12 × 5–10 μ.

Type of Decay.—A brown top and trunk rot. 32 isolations.

Remarks.—Most of the isolates can be readily separated into the fast-growing strain or the slow-growing strain. Occasionally an isolate will be intermediate in respect to growth. The growth rate is not characteristic of isolates from any one host and cultures from cherry belong to both groups. The cultural characteristics of *Fomes pinicola* have been described by Campbell (3) and Mounce (14), both of whom found considerable variation in the growth rates of different isolates.

POLYPORUS BALSAMEUS PK.

(Fig. 6, D, and Fig. 7, B)

Key Pattern.—A–O–M–1–2–10 and E–O–M–1–2–10.

Growth Characteristics.—Growth medium forming in 14 days a mat 5–7 cm. in diameter; mat white to agar-colored, often "Pinkish Buff" to "Cinnamon Buff" around the inoculum; short-cottony, fragile, homogeneous, azonate; margin faintly white, fimbriate.

Hyphal Characteristics.—Both submerged and superficial hyphae staining with eosin, up to 7 μ in diameter, clamps abundant; chlamydospores numerous, entire mat occasionally breaking down into a mass of spores, ellipsoid to barrel-shaped, 9–12 × 6–12 μ, with a hyaline wall and a yellow or brownish content.

Type of Decay.—Brown butt rot. Two isolations.

Remarks.—*Polyporus balsameus* is usually associated with a rot of balsam fir (13)

and other softwoods but has been collected on yellow birch and black cherry on the Allegheny National Forest. The fungus has been described in culture by Fritz (12) who noted the characteristic brownish chlamydospores.

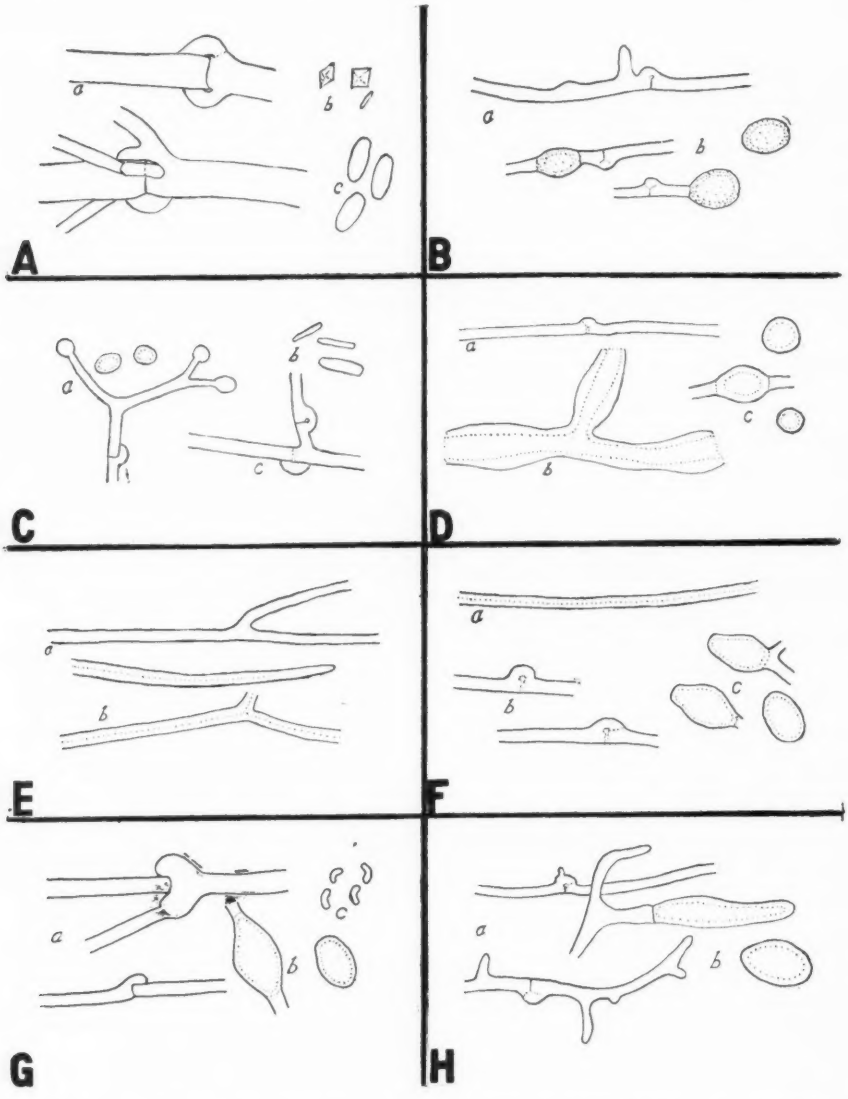

FIG. 7. A. *Coniophora cerebella:* a, hyphae with multiple clamps; b, crystals; c, oidia. B. *Polyporus balsameus:* a, thin-walled hypha; b, chlamydospores. C. *Polyporus subcartilagineus:* a, thin-walled hypha and chlamydospores; b, crystals; c, branched hypha with clamps. D. *Poria mutans:* a, thin-walled hypha; b, large irregular thick-walled hypha; c, chlamydospores. E. *P. prunicola:* a, thin-walled hypha; b, thick-walled hyphae. F. *P. serecio-mollis:* a, thick-walled hypha; b, thin-walled hyphae with clamps; c, chlamydospores. G. *P. xantha:* a, thin-walled hyphae with clamps; b, chlamydospores; c, basidiospores. H. *Trametes serialis:* a, thin-walled hyphae; b, chlamydospores.

POLYPORUS SUBCARTILAGINEUS OVERH.

(Fig. 6, F, and Fig. 7, C)

Key Pattern.—A–O–S–1–2–10.

Growth Characteristics.—Growth slow, forming in 14 days a mat 3.5–5 cm. in diam-

eter; mat white, loose-cottony with fine water droplets on aerial hyphae, very fragile, azonate; margin even, white.

Hyphal Characteristics.—All hyphae staining with eosin, 2–5 μ in diameter; clamps common; chlamydospores few, most abundant on inoculum of 14-day-old cultures, globose or ellipsoid, 5–10 μ in diameter.

Type of Decay.—Brown butt rot. 3 isolations.

Remarks.—This fungus is reported by Overholts (16) from *Picea* and *Prunus serotina* (Fig. 3, D). A similar fungus was also collected on *Liriodendron* by R. W. Davidson and K. D. Doak at Washington, D. C., which is culturally closely related to the fungus on black cherry. *Polyporus subcartilagineus* is a little-known species and no information is available as to its host preferences.

PORIA MUTANS PK.

(Fig. 6, G and H, and Fig. 7, D)

Key Pattern.—B–P–I–1–2–11.

Growth Characteristics.—Growth moderately rapid, mat completely filling Petri dish in 14 days; central zone "Light Ochraceous Buff" to "Ochraceous Buff" and occasionally as deep as "Ochraceous Orange," irregular radiating or indefinite, appressed fine-woolly; rest of mat and marginal portions white or faintly white, appressed fragile usually with a belt of tufted mycelium near the edges of the dish.

Hyphal Characteristics.—Hyphae staining with eosin 2–4 μ in diameter, with many clamps spaced at short regular intervals on the hyphae, only occasionally branched at the clamps; fibrous hyphae at first with slightly thickened walls and staining content, gradually becoming empty with thick, even, or irregular walls, these hyphae prominent and characteristic 4–10 μ in diameter, with occasional cross-walls but no clamps; chlamydospores abundant or rare, mostly globose with a thick hyaline wall, up to 15 μ in diameter; colored hyphae from inoculum coated with fine crystalline material.

Type of Decay.—A white-piped heart and sap rot, usually associated with extensive trunk injuries. Eighteen isolations.

Remarks.—The relationship of this fungus to cultures of *Polyporus croceus* Pers. ex Fr. (10) has not been critically studied although they are very similar in appearance. It is possible that some of the *P. croceus* Pers. ex Fr. cases reported for oak are *Poria mutans*. So far as known the decays produced by these two fungi are also similar. *P. mutans* sporophores were found several times on old cherry logs which contained typical decay.

PORIA PRUNICOLA (MURR.) SACC. AND TROTT.

(Fig. 6, I, and Fig. 7, E)

File Pattern.—C–P–M–11 and D–P–M–11.

Growth Characteristics.—Growth medium, forming in 14 days a mat 5–7 cm. in diameter; mat "Tawny Olive" and "Buckthorn Brown" with a narrow "Antimony Yellow" border 2–3 mm. wide next to the margin; moderately raised, azonate, with a fine, even, woolly surface, loose-felty in texture and peeling cleanly from the agar, not at all tough or leathery; margin proper white or slightly yellowish, narrow, 2–5 mm. wide, even, radiating short-cottony.

Hyphal Characteristics.—Hyphae staining with eosin 1–4 μ in diameter, branched with few cross-walls and no clamps, nonstaining hyphae yellow or dark brown, smooth or irregular, occasionally with slightly swollen spots, with cross-walls and branched, 1–4 (–5) μ in diameter.

Type of Decay.—A red mottled rot of the heartwood. In the incipient stage characterized by a darkening of the normal reddish heartwood. As the decay advances white pockets of completely decayed wood appear in the darkened heartwood. In the advanced stages the wood becomes soft with a white and dark reddish mottled appearance. Sixty-two decay cases.

Remarks.—No fruiting of the fungus was encountered on living infected black cherry and only one sporophore was collected on a down log of the species in the areas studied. Fruiting of *Poria prunicola* was very common on dead fire cherry. *Poria prunicola* sporophores cannot be separated on a morphological basis with certainty from *Fomes igniarius* var. *laevigatus* (Fr.) Overh. or *Poria spiculosa* Campb. & Davidson. These three species are distinct, however, in host relations and cultural characteristics (1, 5, 6).

PORIA SERICEO-MOLLIS (ROM.) BAXTER

(Fig. 6, J, and Fig. 7, F)

Key Pattern.—A–O–M–1–2–11 and E–O–M–1–2–11.

Growth Characteristics.—Growth medium, forming in 14 days a mat 8 to 9 cm. in diameter; mat white or "Pale Pinkish Buff," fragile, fine-woolly to appressed cottony,

azonate or faintly zonate; margin appressed, colorless, even or fimbriate. Mats older than 14 days usually "Pale Pinkish Buff" to "Light Pinkish Cinnamon," cottony.

Hyphal Characteristics.—Hyphae staining with eosin 2–5 μ in diameter, with clamps; nonstaining fibrous hyphae 2–5 μ in diameter, colorless; chlamydospores few or many, lemon-shaped to globose or ellipsoid, 10–16 × 7–12 μ; basidiospores commonly produced in pores, which develop on the upper part of slant on tube cultures, short cylindric 5–7 × 3–4 μ.

Type of Decay.—Brown cubical rot. Ten isolations.

Remarks.—*Poria sericeo-mollis* sporophores have been noted on badly decayed logs of black cherry and also on large open wounds on living trees. Isolations of this fungus were most common from rot associated with large wounds such as those caused by the splitting of forked stems. The interpretation of the species is that given by Overholts (17).

PORIA XANTHA (FR. EX LIND) CKE.

(Fig. 6, K, and Fig. 7, G)

Key Pattern.—A–O–I–1–2–11 and B–O–I–1–2–11.

Growth Characteristics.—Growth moderately rapid, mat completely filling a 9-cm. Petri dish in 14 days; mat white or "Sulphur yellow," azonate, thin, fragile, appressed around the inoculum with scant aerial mycelium, fine-woolly near margin and often forming considerable loose-cottony aerial mycelium against the sides of the dish.

Hyphal Characteristics.—Hyphae staining with eosin 2–8 μ in diameter, clamps common, often double; the larger hyphae finally become empty with a thick hyaline wall or develop the hyaline wall and retain a narrow, irregular lumen, which stains with eosin; chlamydospores, few or many, globose to lemon-shape, with a narrow hyaline wall; an occasional isolate fruits in culture producing allantoid basidiospores 4–6 × 1.5–2.5 μ.

Type of Decay.—Brown cubical rot. One isolation from a badly rotted stump upon which the fungus was fruiting.

Remarks.—*Poria xantha* is common on softwoods (17); often producing decay in structural timbers. Isolates from black cherry, white pine, and hemlock all had the same cultural characteristics.

TRAMETES SERIALIS FR.

(Fig. 6, L, and Fig. 7, H)

Key Pattern.—A–O–M–1–2–11.

Growth Characteristics.—Growth medium, forming in 14 days a mat 6–7 cm. in diameter; mat usually with a tough, white, appressed, occasionally considerably thickened central zone and a wide, faintly white or colorless, appressed, thin, fragile marginal zone; margin proper colorless, appressed, fimbriate. Central zone at times with radiating irregularly spaced ridges and usually with very little loose surface mycelium.

Hyphal Characteristics.—Hyphae staining with eosin 2–5 μ, clamps abundant; fibrous nonstaining hyphae 2–4 μ diameter; chlamydospores few or many, often irregular in shape, 9–18 × 7–10 μ; immature basidia also formed in some isolations in 14 days.

Type of Rot.—Brown rot. Five isolations.

Remarks.—This is not the fungus described from lumber by Cartwright and Findlay (7) and not the same as the culture mentioned by Baxter (2) in his discussion of *T. serialis* and closely related forms. In northern Pennsylvania this form occurs also on other hardwoods and on hemlock.

SUMMARY

A study of cull caused by decay was made in 3 merchantable stands of black cherry on the Allegheny National Forest. Numerous infections, mostly in the butts, were present in 52-year-old trees; these, however, caused only 2.3 per cent cull based on board-foot volume. In two old-growth stands (116 and 120 years old), one of almost pure dense cherry and the other open-grown mixed hardwoods, cull from top or trunk infections was more important than butt rot. In the open-grown 120-year-old trees there was 6.1 per cent cull and in the pure dense stand there was 11.3 per cent cull from decay.

The most important butt rots were caused by *Polyporus spraguei, P. berkeleyi,* and *Coniophora cerebella.* The most important trunk rots were those caused by *Poria prunicola, P. mutans, Fomes pinicola* and *Polyporus*

sulphureus. These trunk rotters entered the trees principally through large branch stubs, except *Poria mutans*, which was usually associated with large wounds.

Most of the rots of black cherry present no external evidence of their presence, except *Fomes pinicola*, which formed conks on half of the trees infected by it.

Twenty-two species of decay-producing fungi were isolated from 212 rot infections in black cherry. Those most frequently isolated were: *Poria prunicola* (62 times), *Fomes pinicola* (32), *Polyporus sulphureus* (28), *Poria mutans* (18), *Polyporus spraguei* (14), *Poria sericeo-mollis* (10), *Polyporus berkeleyi* (9), *Coniophora cerebella* (8), *Trametes serialis* (5), and *Poria inflata* (5).

Good quality black cherry can be grown to large sawlog size without excessive cull from decay. Management to increase diameter growth after the early height growth is attained should hold down the amount of cull from decay if the trunks of the reserved trees are not injured severely.

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THE FUNGUS CAUSING THE SO-CALLED "SEPTORIA LEAF-SPOT DISEASE" OF RASPBERRY

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(Accepted for publication April 1, 1943)

Infection characterized by small, angular to circular, grayish lesions on living leaves of raspberry, commonly known as Septoria leaf-spot disease, is prevalent in most regions in the United States. It is the most destructive fungus disease of raspberry in the Middle Atlantic States and westward to Arkansas. A similar disease, caused by *Septoria rubi* West., attacks the *Eubatus* group of *Rubus* and is prevalent in almost every region where members of this group are found.

For almost a century mycologists and plant pathologists generally have considered *Septoria rubi* or one of its described varieties to be responsible for the disease in all species of *Rubus*. In 1921 an ascogenous stage of the fungus, *Mycosphaerella rubi*, was named and described by Roark (15) from overwintered leaves of both raspberry and blackberry.

In 1939 the writers observed that a *Sphaerulina* occurred abundantly on overwintered raspberry leaves at Beltsville, Maryland, and found that it was a stage in the life cycle of the common raspberry leaf-spot fungus, although it failed to infect leaves of blackberry and dewberry. These observations led to a more extensive study of the fungi causing the so-called Septoria leaf spot of *Rubus* spp., and the findings are herein reported. The evidence indicates that the fungus attacking raspberry and having a *Sphaerulina* perfect stage attacks neither blackberry nor dewberry.

REVIEW OF LITERATURE

Reference in literature to the raspberry fungus is very meager. In 1885 Ellis and Morgan (in a paper by Ellis and Everhart (8)) published as new the species *Cylindrosporium rubi* found on living leaves of the black raspberry (*Rubus strigosus* Michx.), from a collection apparently made by J. J. Brown of Wisconsin. Their description of the disease agrees in general with observations made by the writers of the raspberry disease and fungus now under consideration. Furthermore, a specimen in the Mycological Collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering, which is undoubtedly a part of the type collection of *C. rubi* collected by J. J. Brown in Wisconsin in 1885, was examined by the writers, who believe it to be identical in every respect with the common raspberry fungus heretofore referred to as *Septoria rubi* West.

Zundel (23), in 1942, reported *Cylindrosporium rubi* as causing widespread damage to red raspberries in Pennsylvania.

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It is not clear just when *Septoria rubi* was first reported as associated with a raspberry disease similar to that of the blackberry. Galloway (9) reported upon "experiments in the treatment of *Septoria* of raspberry and blackberry" as early as 1891. In Rabenhorst's Krypogamen Flora, Allescher (1), in 1901, lists raspberry, in addition to blackberry and dewberry, as a host of *S. rubi.* Bennett (3) mentioned that "raspberry, dewberry, and blackberry are attacked by a rather common type of leaf spot." Colby, Anderson, and Flint (5) wrote: "On raspberry leaves these spots soon turn to ashen-gray in the center. On blackberry and dewberry leaves the edge of the spots has a zone of purple tissue and the center is light brown to tan in color. . . . The disease is caused by a fungus, *Mycosphaerella rubi.* It is more commonly known under the old name *Septoria rubi* which was applied before the perfect stage was known." Dodge and Wilcox (7) stated that "leaves of red raspberry are frequently attacked by the same fungus that causes leaf spot of dewberry." Hesler and Whetzel (10) reported that "the common leaf spot of blackberry affects also raspberry and dewberry." Roark, in the opening statement of his paper describing the perfect stage of the Rubus *Septoria*, said: "The fungus formerly known as *Septoria rubi* Westendorp causes leaf spots on many species of *Rubus.* . . ."

The blackberry leaf-spot fungus was given the name *Septoria rubi* by Westendorp (19) in 1854, when issued in Westendorp and Wallay's Herbier Cryptogamique Belge, Fasc. 19–20, No. 938, and accompanied by label with description. He gave leaves of *ronce* (briar, bramble) as the host. The pycnospores (*sporidies*) were described as cylindrical, rounded at both ends, with 3 to 5 segments (sporules) very difficult to see, 1/100 to 1/200 mm. long. There seems to be some gap in the early taxonomic history of the fungus. On the label of the specimen two synonyms are given as follows: "*Sphaeria rubi* Duby, Bot. Gall II, p. 712 ?—*Depazea rubi* West. et Vanhaes, Cat. Crypt., No. 80." The question mark after "p. 712" may mean that Westendorp was uncertain as to the synonymy. The writers have not been able to find in literature and exsiccata available for examination any reference to *Depazea rubi.*

Kickx (12) in 1867 (p. 453) gave a fuller description of the fruiting body and the leaf manifestations of the fungus, but used Westendorp's description and measurements of the pycnospores.

Saccardo's (17) description of the fungus and the spots on the leaves is similar to the descriptions by Westendorp and by Kickx, except that he recorded the pycnospore dimensions as 40 to 55 μ long and 1.5 μ wide.

Berkeley and Curtis (4), apparently not knowing of Westendorp's *Septoria rubi*, gave the same name in 1878 to a fungus on Rubus leaves collected along the Santee River in South Carolina. Of course Westendorp's name has priority over *Septoria rubi* Berk. and Curt.

Several other references to *Septoria* on *Rubus* are recorded in literature; 2 forms are designated as distinct species and others as varieties of *S. rubi.* Descriptions of these are either meager, or based upon the color of the

resulting lesions, or the pycnospores are shorter than the usual concept of *S. rubi* West. In the opinion of the writers, length of the pycnospores alone cannot always be used as a basis for separating species or variants. They have found that the pycnospores, especially those on raspberry, vary considerably in the same pycnidium. Those formed immediately after maturity of the pycnidia are longer and more normal than those at the end of the period of pycnospore formation. Old pycnidia, once spent or exhausted, will contain a few short and otherwise abnormal pycnospores. It is thought that some descriptions of *Septoria* on *Rubus* have been made from material too old for satisfactory diagnosis.

Septoria comitata J. J. Davis was described from Caeoma-infected leaves of *Rubus allegheniensis*. The description does not indicate the fungus to be different from *S. rubi* West. Davis (6) made the following notation in his description: "While it is possible that this is a form of *S. rubi* West., modified by the form of the substratum, it is being kept separate under the name *Septoria comitata* n. sp. ad interim." The following varieties of *Septoria rubi* West. have been described as occurring on either blackberries or dewberries: Var. *pallida* Ell. and Holway; var. *brevispora* Sacc.; var. *saxatiles* Allescher; and var. *asiatica* Bub. There also has been described var. *alba* Pk. of *Septoria rubi* Berk. and Curt. The first two were separated because of their short pycnospores. The writers examined a portion of the original collection of Saccardo's variety *brevispora* kindly sent to them by H. D. House, curator of the New York State Museum. Their measurement of pycnospores from this material showed them to vary from 22–48 μ by 1.6–3 μ. This is not greatly out of line with the normal dimensions of *S. rubi* West. in blackberry.

It seems that the fungus also has been designated as *Ascochyta rubi* Lascher (13), *Rhabdospora ramealis* (Desm. and Rob.) Sacc. (18), and *Sphaerella ligea* Sacc. (16).

In 1937, Zeller (21), after examining specimens of *Septoria*-infected leaves of *Rubus* from various localities, concluded that there are 2 Septoria leaf-spot diseases of *Rubus* in the United States and reported: "The materials indicated that the leaf spots from the Pacific Northwest, Wisconsin, Durham, North Carolina, and England are essentially alike and constitute the usual concept of *Septoria rubi* West. The leaf-spot material sent from Beltsville, Maryland, and Willard, N. C., however, had a different appearance." He further reported that the Beltsville material proved to be a fungus first described by Saccardo as *S. rubi* West. var. *brevispora* from material on leaves of *Rubus hispidus* collected by C. H. Peck at New Chatham, New York. Zeller believed that there were so many distinct physiological and morphological differences between *S. rubi* West. and var. *brevispora* Sacc. that one could not be considered a variety of the other, and he, therefore, raised Saccardo's variety to specific rank and proposed the new combination *S. brevispora* (Sacc.) Zeller. Later, learning that the binominal *S. brevispora* was preoccupied, he applied the new name *S. darrowi* (22).

Zeller was not very specific as to the host on which he found his *Septoria darrowi* (*S. brevispora*), but considered it broadly as a fungus parasitic on the genus *Rubus*. He referred to what one would expect to be his type specimens as the "Beltsville, Maryland, material." That could have been one of several species of blackberry, dewberry, or raspberry. He, however, illustrated his fungus by a drawing of a pycnidium and pycnospores from a raspberry leaf and showed for comparison a drawing of *S. rubi* in Himalaya blackberry (*Rubus procerus*). He described his fungus as characterized by small pycnidia (27–35 μ high and 46–60 μ broad) with pycnospores 15–30 × 1.8–3.4 μ. Presumably, he was describing the raspberry fungus. If so, the name *Cylindrosporium rubi* Ell. and Morg. should have priority over Zeller's *S. darrowi* in absence of proof of their dissimilarity.

The short pycnospores recorded by Zeller could have been attributed to the condition of his specimens. If his specimens were collected in autumn and the normal pycnospores had been discharged and washed or weathered out, then he would have had for measuring only the abnormal, short, blunt, 1- to 3-septate pycnospores in the spent pycnidia.

From the descriptions it would appear that *Septoria rubi* Berk. and Curt., *S. comitata* J. J. Davis, and all varieties of *S. rubi* should be considered as synonyms of *S. rubi* West.

There seems to be some significance in the fact that all species of Septoria on brambles have been recorded as pathogens in "*Rubus*" or in some blackberry species; in no case in any original description has the raspberry been mentioned specifically as a host.

THE RASPBERRY PATHOGEN

The Pycnidial Stage

The raspberry leaf-spot fungus is parasitic in living leaves and in the thin cortical layer of canes, and forms pycnidia and perithecia in fallen leaves during fall and winter.

On the green leaves, the immature fruiting bodies show faintly under the upper epidermis and within the boundary of small greenish-black spots (Fig. 1, A) about as soon as the spots are perceptible. Development of the pycnidia proceeds rapidly. They mature and pycnospores are produced and discharged before the fungus-invaded tissues are completely necrotic, as is evidenced by a color change from greenish-black to gray (Fig. 1, B).

The pycnidia are subglobose to ovoid, average about 70 μ high and 80 μ wide, and lie within the palisade region of the leaf structure (Fig. 1, C) with their base extending well into the spongy parenchyma. In the early stages of its development, before the initiation of pycnospores, the pycnidial wall consists of a thin layer of closely interwoven hyphae. At maturity the base and side comprise 1 to 3 layers of thin-walled cells, and the top is covered with 1 layer, devoid of any ostiole structure. Apparently, the pressure exerted from within by the production of a large quantity of pycnospores is sufficient to rupture the pycnidial covering and the over-lying epidermis.

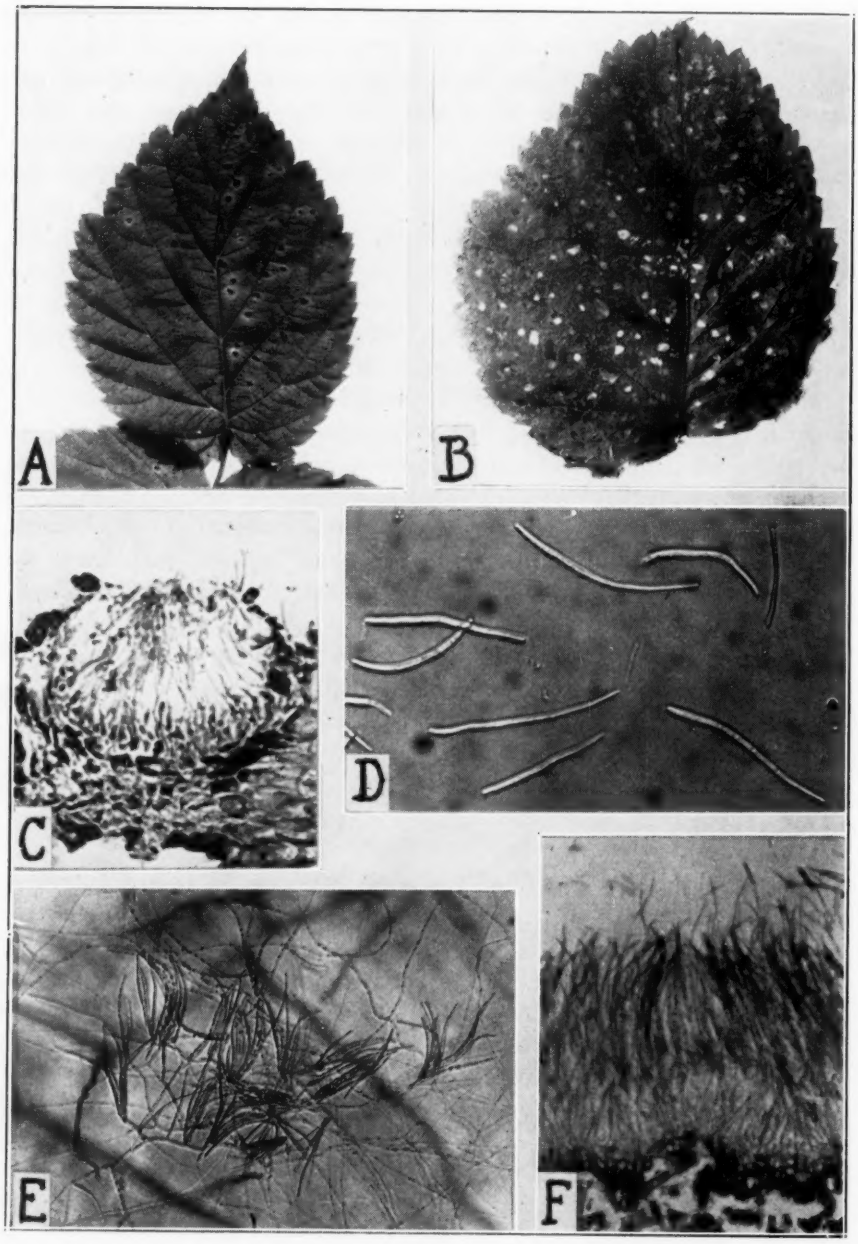

FIG. 1. A. Young raspberry leaflet showing early stage of the disease, when the pycnidia are discharging pycnospores. B. Leaflet showing older and more conspicuous stage of spotting, after the pycnospores have been discharged. C. Pycnidium from a living leaf. ×350. D. Pycnospores from a leaf pycnidium. ×375. E. Conidia formed on hyphae in young colony on corn-meal agar. ×125. F. Heavy production of conidia from a sporogenous layer in an old corn-meal agar culture. ×350.

The pycnospores are discharged as an aggregate, with the shape of a horn or cylinder, and having a diameter about equal to that of the fruiting body, which becomes hard when dry. Dew or rain dissolves the matrix cementing the pycnospores. The pycnospores may later be washed away, leaving the empty pycnidium surrounded by a ring of ruptured epidermis, which then appears as a tiny crater.

The pycnospores develop from short sterigmata growing from the sides and base of the pycnidium. The pycnospores are elongate, usually curved, slightly obclavate, thickest about one-third the distance from the base, and tapering toward the apex (Fig. 1, D). They are hyaline, granular, 3- to 9-septate, and average 55 μ long and 3.5 μ wide. Although the length varies from 32 to 86 μ, the great majority of the pycnospores fall within the range of 40 to 60 μ. Measurement of width of pycnospores, made through region of greatest dimension, varies from 3.0 to 4.8 μ. Germination takes place terminally and laterally.

The fungus commonly infects the canes of the raspberry and usually shows only as scattered pycnidia on the surface of the lower portion of the canes, or infrequently causes discolored or necrotic areas in the cortical tissues. Because cane infections do not always cause conspicuous lesions, infected canes probably occur more commonly than is ordinarily suspected; in fact, pycnidia frequently were observed on canes of raspberry varieties whose leaves are susceptible to infection. The fungus on the canes was demonstrated by inoculation experiments to be identical with the fungus on the leaves (see page 999). This fungus is thought to be the same as described by Ellis (published by George Martin (14)) from raspberry canes and named *Rhabdospora rubi*. The pycnidia, although showing on the canes during late fall and winter, do not sporulate until May or June and are undoubtedly an important source from which new spring leaves become infected. Pycnidia on planting stock may serve to distribute the fungus from one locality to another (Fig. 2, F).

The cane pycnidia are subepidermal and partly imbedded within the cortex (Fig. 2, G). They vary from subglobose-ovoid to lenticular, and have a broad ostiole through which the conidia are discharged in the form of a waxy cirrus. These fruiting bodies are more typical of the usual concept of a pycnidium than those that develop in living raspberry leaves.

The pycnospores in most respects resemble those formed during the summer in leaves. The cane form is shorter, averaging 40.8 μ long, but has a width equal to those in the leaves. The most significant difference noted is that there are fewer showing the obclavate shape. Most of them are uniformly cylindrical from the base to the tapering apical cell.

Soon after the infected leaves fall, winter pycnidia begin to develop near the old lesions. These show on both surfaces but in greater number on the underside, where they are concealed by the dense tomentum, unless it is wet. These pycnidia may be found at any time from late autumn to spring. They are at first subepidermal but emerge later through the epidermis and appear

to be quite superficial. They are black, subglobose to urn-shape and measure about 80–121 × 65–101 μ, averaging 94 to 82 μ (Fig. 2, A, a). The side of the pycnidial wall is composed of 2 to 4 layers of thick-walled cells.

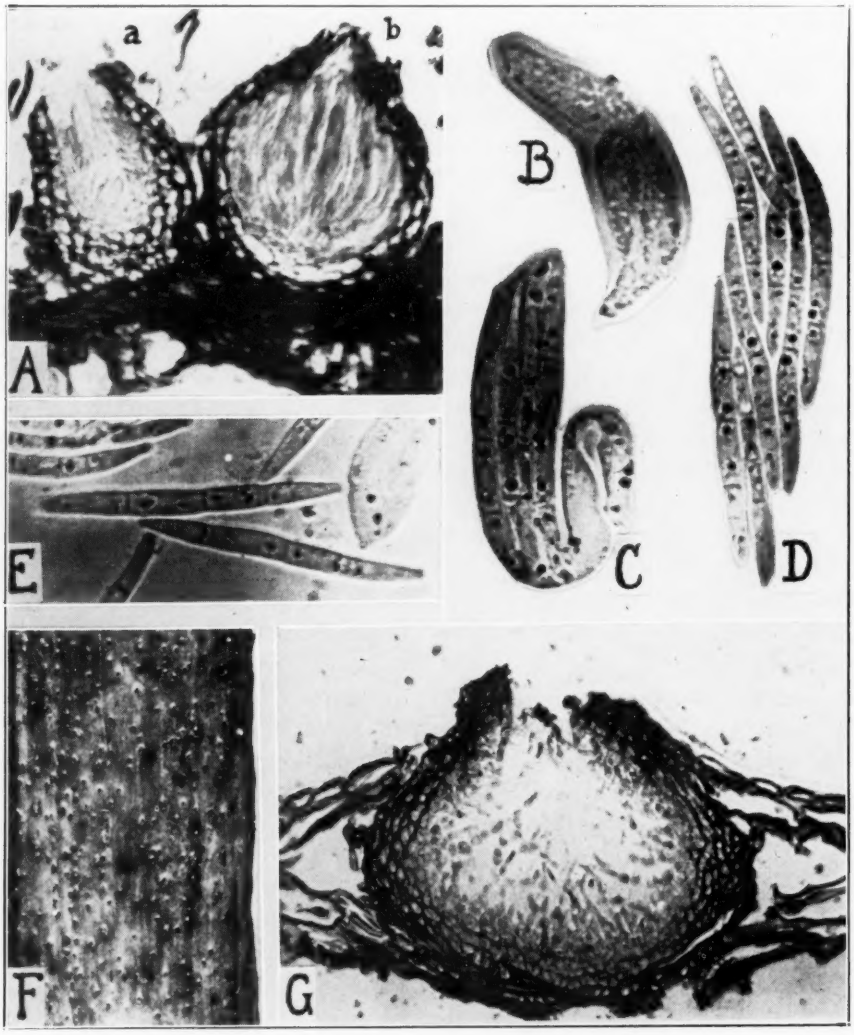

FIG. 2. Winter stage of the raspberry leaf spot fungus. A. A pycnidium (a) and a perithecium (b) of the *Sphaerulina* on an overwintered leaf. × 350. B. An ascus as usually seen in freshly mounted specimens. C. Developing ascospores being discharged from a ruptured ascus, but still retained by an inner ascus membrane. × 800. D. Fully developed ascospores immediately after being liberated from an ascus. × 800. E. Ascospores showing characteristic septation. × 800. F. Raspberry cane showing scattered distribution of the winter pycnidia. × 4. G. A winter pycnidium from a cane. × 350.

Stained sections do not show clearly the presence of an ostiole, and the weight of evidence indicates that an ostiole is lacking.

The winter pycnospores are in all essentials like those produced on green leaves. The cultural characters of the overwintering fungus are identical with those of the summer form. The presence of both pycnidia and perithecia on overwintered, diseased leaves, with an apparent cellular connection (Fig. 2, A, a and b) strongly suggests genetic relationship. Proof of such relationship by inoculation was not attempted.

THE ASCOGENOUS STAGE

In the spring of 1939 a search was made for the ascogenous stage of the common Septoria on overwintered leaves of blackberry, dewberry, and raspberry. According to Roark (15) the perfect stage is a *Mycosphaerella*. No fungus resembling that described by Roark was found on any leaves examined, although perithecia of the *Sphaerulina* type were commonly observed on raspberry leaves. This fungus was assumed to be some saphrophyte, hence no further study of it was then made.

The following spring another search was made for the *Mycosphaerella* on *Rubus*. *Sphaerulina* was again plentiful on overwintered raspberry leaves. Two collections of dewberry leaves were made that bore perithecia of the *Mycosphaerella* type (cultures from these perithecia proved later not to be parasitic on *Rubus*); and of several collections of overwintered leaves of blackberry none had perithecia of any kind.

Ascospores from perithecia of the Sphaerulina type of the raspberry fungus were isolated and grown on corn-meal agar; the resulting growth of

TABLE 1.—*Results of direct and cross inoculation of leaves of raspberry, dewberry, and blackberry with pathogens isolated from each form*

Source of cultures used for inoculum	Plant inoculated	Number of plants inoculated	Number of leaves inoculated	Number of leaves infected	Number of lesions appearing
Pycnospores from living raspberry leaf	Raspberry	10	85	75	22,350
	Dewberry	11	99	0	0
	Blackberry	11	90	0	0
Sphaerulina ascospores from overwintered raspberry leaf	Raspberry	10	132	121	18,300
	Dewberry	4	67	0	0
	Blackberry	3	24	0	0
Pycnospores from living dewberry leaf	Dewberry	5	54	35	1,490
	Blackberry	10	104	79	450
	Raspberry	5	47	0	0
Pycnospores from living blackberry leaf	Blackberry	11	79	53	1,180
	Dewberry	7	51	19	275
	Raspberry	7	62	0[a]	0[a]

[a] Two plants infected. See text, p. 999.

hyphae and conidia resembled the fungus previously isolated by using pycnospores of the leaf-spot fungus from living raspberry leaves. A water suspension of the conidia from ascospore isolates was applied to the foliage of disease-free potted raspberry plants in a greenhouse. In about 2 weeks lesions appeared on the inoculated leaves. In these lesions occurred fruit-

ing bodies and pycnospores similar to those of the fungus naturally present on raspberry leaves. During the following 2 years, numerous successful inoculations were made on raspberry leaves with conidia from cultures originating from single-ascospore isolations of the raspberry *Sphaerulina*. Parallel inoculations on dewberry and blackberry leaves were negative. The results of these inoculations are given in table 1.

The Perithecia

Perithecia form in late fall; asci develop only on resumption of warm spring weather. The fungus, somewhat sensitive to weather conditions, does not complete its growth cycle if weather is too hot or too dry. Perithecia are largely superficial, with bases embedded in spongy parenchyma, and protrude through broken epidermis. They are black, ovate to conical, slightly papillate, 88–140 μ high, and 86–120 μ wide (Fig. 2, A, b). The perithecial wall is thin, comprising 2 to 3 layers of cells.

Development of the asci and ascospores was observed over a period of several hours after mounting in a drop of tap water inverted over a Van Tieghem cell. Usually, when asci were first separated from the perithecium, they appeared immature (Fig. 2, B), with homogenous and granular contents; but, within 2 hours, faint lines representing the 8 ascospores began to show (Fig. 2, C). The development of the ascospores continued slowly until their form was seen clearly. The ascospores at this stage were packed within the ascus without any apparent order. Simultaneously, with the development of ascospores, the ascus slowly distended 20 to 30 μ in the direction of the long axis and at the same time the spores changed position to fill this space (Fig. 2, C). What happens to the ascus wall is not clearly understood, but apparently it ruptures horizontally and the tip is pushed upward or to one side by the pressure of the developing spores. An inner membrane, or spore-enclosing, wall disappears and the mature spores drift apart (Fig. 2, D). No indication of their forceful discharge has been observed.

Asci are clavate-cylindrical, with obtuse or somewhat pointed apex; 44.8 μ to 70.4 μ long by 9.6 μ to 15.0 μ wide. The ascus wall is 2–3 μ thick, sometimes apparently thicker at free end; no paraphyses were observed. The ascospores are hyaline, granular, cylindrical, ends somewhat pointed but a little more so at apex, slightly curved, usually 4-celled, less frequently 6–8-celled, 32–57 μ long by 3.5 to 5.8 μ wide (Fig. 2, D and E).

The spores germinate within 1 or 2 hours after liberation from an ascus. Usually a germ tube grows from the free end of each terminal cell. Less frequently the intermediate cells germinate; and when they do, the germ tube emerges near a septum.

The writers made a search for perithecia on overwintered leaves of various form of *Rubus* for 3 successive years. In addition to examining collections taken from different localities, they collected in the autumn spotted leaves of blackberry, dewberry, and raspberry at Beltsville, Maryland. A

portion of these collections were left in the field with only enough protection to prevent scattering by the wind, and marked so they could be found for examination the following spring. Other portions of the collection were placed in floorless wire cages, so that the leaves would be in contact with the soil. At various times during the winter and spring, specimens from the field or cages were transferred to a greenhouse or moist chamber in the laboratory. The *Sphaerulina* was found on raspberry leaves each year without difficulty.

Only infrequently have perithecia of any kind been observed on overwintered blackberry and dewberry leaves. Perithecia of *Mycosphaerella* type were observed on a few collections taken near Beltsville, Md., and near Rose Hill, Willard, and Raleigh, N. C. The size of the asci and ascospores, and character of the growth on artificial media indicated that 3 groups or species were represented. One type, collected in 1941 on dewberry at Rose Hill, N. C., and in 1942 at Beltsville, Md., produced small, hyaline, 1-septate ascospores, the 2 cells being of unequal size. The spore measurements varied from 6.8 to 12.0 μ long by 2.6 to 3.8 μ wide. Twenty-seven single-spore isolates were made from this form. The colonies on artificial media did not sporulate and were otherwise unlike any fungus isolated from living *Rubus* leaves.

A second form, also bearing ascospores with cells of unequal size, was collected on overwintered dewberry leaves at Beltsville. These spores ranged from 12.0 to 16.0 μ long, by 3.2 to 3.5 μ wide. Thirty-two single-spore isolations were made from the 2 collections. The appearance of this fungus on corn-meal agar somewhat resembled the dewberry leaf-spot fungus. Black, conical, ostiolate pycnidia were formed that extruded masses of cream-colored filiform pycnospores measuring 17.8 to 27.6 μ long and 1.6 to 2.5 μ wide, or about half the length of the pycnospores of the dewberry Septoria. Several attempts were made to infect dewberry and blackberry leaves with this ascospore isolate. Fourteen Lucretia dewberry and 2 Lawton blackberry plants were inoculated. No infection developed. The fungus was considered non-parasitic on the 2 *Rubus* species inoculated and different from *Septoria rubi.*

The form most frequently present on overwintered leaves was collected on dewberry at Beltsville in 1940 and 1942, also at Rose Hill, N. C., in 1941, and on blackberry and dewberry at Raleigh, N. C., in 1942. Ascospores were hyaline, 1-septate, the 2 ascospore cells being about equal in size. Ascospores were 14.4 to 22.0 by 2.8 to 3.5 μ in dimensions. The spores of this fungus are only slightly smaller than the measurements reported by Roark (15) for *Mycosphaerella rubi;* and are apparently identical with those of the single specimen filed in the Mycological Collections of the Bureau of Plant Industry, labeled *Mycosphaerella rubi* (West.) Roark. This specimen was determined by F. A. Wolf from a collection made at Raleigh, N. C., in 1924.

Eighty single-spore isolations were obtained by the writers from the collections taken from the 3 localities mentioned above. The resulting colonies resembled somewhat those of *Septoria rubi*, but grew more rapidly and produced neither conidia nor pycnospores. It is believed, therefore, that this fungus is not the ascogenous stage of *S. rubi* West. The fungus the writers do consider to be *S. rubi* West., which attacks living leaves of the *Eubatus* group of *Rubus*, has been isolated from collections made from various parts of the United States, and in every case has produced either conidia or pycnospores on corn-meal agar.

In Roark's (15) paper on the Septoria leaf spot of *Rubus* the claim is made that the Septoria occurring on *Rubus* is the imperfect stage of a *Mycosphaerella* with ascospores measuring 20 to 25 × 3.5 to 4.25 μ. This *Mycosphaerella* was found by Roark on overwintered leaves of 2 raspberry species, *Rubus strigosus* Michx. and *R. parviflorus* Nutt., and on 2 blackberry forms, *R. allegheniensis* Porter and *R. hispidus* L., in a restricted section of Wisconsin.

As far as is known by the writers no one in the United States except Roark has demonstrated the relationship between a *Septoria* on *Rubus* and a *Mycosphaerella*.

The very excellent original draft of Roark's doctor's dissertation[2] from which the published extract was taken was examined by the writers. The unpublished paper shows that Roark isolated an ascogenous fungus from overwintered raspberry leaves. Subcultures of a majority of these isolations formed conidia (secondary conidia) in abundance. When these were sprayed on raspberry leaves with an atomizer the well known leaf spot resulted; but, when dewberry leaves were inoculated with the conidia, the result was negative. Inoculations made from an ascogenous fungus on overwintered blackberry leaves made, with one exception, nonsporulating colonies on artificial media. Inoculum from the one sporulating culture was applied to 1 Cuthbert red raspberry and 2 Lucretia dewberry plants. Roark's report shows that he secured on 1 dewberry plant "several spots that look like Septoria spots; but no pycnidia." No infection occurred on the other dewberry plant inoculated, nor on the raspberry plant.

Because of the nonsporulating character of his isolates from perithecia on overwintered blackberry leaves, Roark resorted, for inoculum, to fragments of leaves bearing perithecia and depended upon the forceful expulsion of ascospores to the under surface of leaves. Twenty-five such trials were attempted and 2 were considered successful. In one case leaves of *Rubus hispidus*, with an abundance of mature perithecia, were "arranged so that ascospores would be discharged upon the moistened lower surface of cultivated blackberry leaves." The plant was kept under a bell jar for 4 days. On 4 leaflets groups of brown spots developed and, later, 2 pycnidia. Roark stated: "It is possible but not probable that the lesions were caused

[2] Doctor's dissertation entitled "The *Septoria* leaf-spot disease of *Rubus*," by E. M. Roark; deposited in the Library of the University of Wisconsin, Madison, Wisconsin.

by pycnospores from pycnidia present along with the perithecia on the pieces of dead leaves." In another case Lucretia dewberry leaves were inoculated in the same manner and from the same source. The inoculated leaves developed "many very small red spots but these red spots never developed any further."

In summarizing the results of inoculations using isolates from pycnospores as the inocula, Roark (15) stated: "None of the strains isolated from the raspberry group were found to infect any member of the blackberry group, and none of the strains from the blackberry were found to infect any type of raspberry."

Zeller (21), who made a study of *Rubus* leaf-spot fungi, and examined some of Roark's material on which the latter based his *Mycosphaerella*, stated that "Some question arises concerning the perfect stage of *Septoria rubi* West. . . . We have never seen in Oregon any *Mycosphaerella* on *Rubus* similar to *M. rubi* Roark."

The present report concerns principally the taxonomic status of the raspberry fungus, and evidence is presented to show that the imperfect stage is identical with *Cylindrosporium rubi* Ell. and Morg., and that the ascogenous stage is a *Sphaerulina*, apparently not heretofore described, and for which the name *Sphaerulina rubi* is proposed. This fungus is believed to be different from *S. intermixta* (Berk. and Br.) reported (20) as occurring in Europe on dead branches of *Rubus* and *Rosa*, and having short ascospores measuring 16 to 18 μ long or about one-half the length of ascospores of the raspberry *Sphaerulina*.

The following is a description of the raspberry leaf-spot fungus: *Sphaerulina rubi* sp. nov.

Stat. conid: *Cylindrosporium rubi* Ell. and Morg. (8). Perithecia usually numerous, scattered or in groups, mostly hypophyllous, at first subepidermal, later erumpent, black, conical, ostiolate-papillate, 88 to 140 μ high and 86 to 120 μ wide. Asci fasciculate, sessile, clavate-cylindrical, curved or straight, 44.8 to 70.0 μ long and 9.6 to 15 μ wide, containing 8 spores; outer wall about 2 μ thick; inner wall membranous. Ascospores hyaline, granular, cylindrical, usually curved, pointed at both ends, slightly more so at apex, normally 4-celled, less frequently 6- to 8-celled; 32.0 to 57.6 μ long and 3.5 to 5.8 μ wide. Paraphyses lacking. Hab. in overwintered leaves of *Rubus strigosus* Michx. in Maryland, Missouri, and North Carolina.

Perithecia plerumque numerosa, conspersa, vel caespitosa, vulgo hypophylla, primum subepidermicalia dein erumpentia, atra, conica, papillato-ostiolata, 88–140 μ alta, 86–120 μ lata; asci fasciculati, sessiles, clavato-cylindrici, curvati vel recti, 44.8–70 μ longi, 9.6–15 μ lati, octospori; tunica exteriori circa 2 μ crassa, interiori membranacea; ascosporae hyalinae, granulosae, cylindricae, plerumque curvatae, utrinque praecipue ad apicem acutae, typice 4-, rarius 6–8-cellulatae, 32–57.6 μ longae, 3.5–5.8 μ latae; paraphyses nulli. Hab. in foliis anni praeteriti *Rubi strigosi* Michx. Maryland, Missouri, et North Carolina.[3]

Pycnidial stage: (*Cylindrosporium rubi* Ell. and Morg. (Emended)). On living leaves, causing circular to angular spots, at first greenish-black, later grayish, usually 1 to 2 mm., sometimes 4 to 6 mm. in diameter; pycnidia, epiphyllus, subepidermal; 58 to 80 μ high and 58 to 121 μ wide; wall thin, 1 to 3 cells thick; pycnospores elongate, obelavate, slightly curved to falcate, pointed at one end, 3- to 9-septate, hyaline, 32 to 86 μ long by 3.0 to 4.8 μ wide in region of greatest thickness. Common in living leaves of red raspberry, *Rubus idaeus* L., and black raspberry, *R. occidentalis* L., east of the Rocky Mountains. Also occurring in canes and overwintered leaves.

[3] Latin diagnosis prepared by Miss Edith K. Cash, Division of Mycology and Disease Survey, U. S. Department of Agriculture.

Type specimen of the fungus has been deposited in the Mycological Collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Beltsville, Maryland, under No. 71381.

PATHOGENICITY OF THE RASPBERRY FUNGUS

Although these studies pertain primarily to the raspberry leaf-spot fungus, parallel studies of the fungi from leaf spots on blackberry and dewberry were made in order to present a more complete picture of the heretofore confused status of the *Septoria* pathogens attacking *Rubus*.

Single pycnospore isolates were made from the leaf-spot fungi on blackberry, dewberry, and raspberry; likewise, monosporic cultures were made from ascospores of the *Sphaerulina* on raspberry.

Inoculation Experiments

The inoculation work was done on especially grown greenhouse plants. The variety Taylor, the most susceptible one available, was employed for the raspberry studies. The Lucretia dewberry and Lawton blackberry were selected to represent the *Eubatus* section of *Rubus*. The plants, with tops cut below the ground line, were taken from the field in the fall and set in 6- or 8-inch pots. From 1 to 5 disease-free shoots grew from each potted root, and when they had attained a height of 8 to 15 inches, they were considered suitable for inoculating. Each shoot bore leaves of varying age and size, from a closed terminal bud to fully mature leaves. Their size and maturity were recorded on tags attached to each petiole when the several leaves were inoculated. The inoculum, a spore suspension in distilled water with a small amount of Santomerse as a spreader, was applied with an atomizer. The Santomerse proved especially advantageous in case of the raspberries, because of the thick tomentum on the lower leaf surface. It was learned earlier that infection took place principally on the lower leaf surface and that water alone did not readily penetrate the tomentum. Inoculated plants were held for 48 to 72 hours either under bell jars or in a moist chamber equipped with an automatic humidity regulator. Temperature regulation for the inoculated plants during the incubation period was found necessary. The raspberry fungus required a somewhat higher temperature for germination and growth than did the blackberry and dewberry fungi. These temperature requirements were met by holding the inoculated plants during the incubation period in greenhouses maintained at the required temperature levels.

For computing the degree of infection the entire leaf, instead of the individual leaflets, was used as the unit. When the raspberry fungus was inoculated on leaves of its host, heavy infection usually resulted. This fungus at no time ever infected either blackberry or dewberry leaves. A large number of lesions appeared after inoculating blackberry leaves with the blackberry isolate or dewberry leaves with the dewberry form. Cross-inoculation with these 2 latter strains on the 2 hosts concerned resulted in

fewer infections than when the isolates were put on their own hosts. The dewberry isolate was never induced to infect raspberry.

The 7 raspberry plants inoculated with the blackberry fungus (Table 1) were of normal size for the purpose, the foliage ranging from unopened buds to old and mature leaves. The blackberry fungus apparently did not infect such leaves, but did attack very small ones on a few other raspberry plants that were abnormal. These plants, having grown too large for convenient handling, were cut back to the ground line. As a result numerous short, weak shoots with very small leaves developed from the underground parts. The limited number of spots observed on these leaves averaged about $\frac{1}{2}$ mm. in diameter, and had 1 pycnidium in each spot. Size and shape of the pycnospores, growth on artificial media, and inoculation on blackberry leaves proved this fungus to be identical with the one ordinarily inhabiting blackberry leaves, and not the one naturally parasitic on raspberry leaves. This experiment was repeated at another time with similar results.

Since the pycnidia commonly found on raspberry canes contain pycnospores similar to those produced on living raspberry leaves, it was thought essential to determine whether or not a relationship exists. Single-spore cultures were made of the cane fungus, and the resulting conidia were applied to raspberry leaves in the usual manner. A total of over 500 lesions developed on 37 of the 50 inoculated leaves. Raspberry plants inoculated with spores of the leaf pathogen developed pycnidia on the canes when held over winter.

Pathogenicity of the ascogenous stage of *Sphaerulina* from overwintered raspberry leaves was demonstrated by inoculating 132 raspberry leaves with conidia from cultures derived from ascospore isolates. A moderately heavy infection of the leaves was obtained. From the number of leaves inoculated, 121 showed a total of over 18,000 spots within 17 to 24 days. This demonstration is thought to be adequate proof of the genetic relationship of the *Sphaerulina* with the raspberry leaf-spot fungus heretofore generally referred to in literature as *Septoria rubi* West. The conidia from the ascospore isolates failed to infect leaves of blackberry and dewberry inoculated under conditions similar to those under which the raspberry plants were held.

Age and Surface of Leaves Most Susceptible to Infection by the Raspberry Fungus

Since the leaves on each inoculated plant were tagged, described, and numbered on the date of inoculation, it was possible upon completion of the observation, 3 or 4 weeks later, to determine the approximate age, size, and condition of leaves most susceptible to infection.

The size, or condition, of the leaves was classified as 1, the terminal bud; 2, unfolding buds; 3, small, but expanding leaves; 4, leaves $\frac{1}{2}$ grown; 5, full-size leaves, yet immature, somewhat succulent and greenish-yellow; and 6, leaves considered as mature—that is, fully grown, dark green, with firm texture.

These data, involving 256 leaves and over 37,000 lesions, show that only the young leaves are susceptible to infection. The greatest susceptibility of the leaf seems to be during that period when the leaf area is still increasing, and the tissues are succulent. Very small leaves appear to be less susceptible than those a few days older, but this may be due to the smaller area for collecting spores. After the leaves were fully grown and had developed their normal dark green, they seldom became infected in the inoculating chambers.

In the experiments planned to determine which surface of the leaf is more subject to infection, the inoculum was applied by means of a camel-hair brush. It was shown that infections ordinarily occur on the lower surface of the leaves of raspberry (Table 2). The occasional spots that appear when the inoculum is applied to the upper surface are attributed either to faulty technique or injury of the upper epidermis.

TABLE 2.—*Results of inoculating under and upper surfaces of raspberry leaves with pathogens isolated from this species*

Source of cultures used for inoculum	Plant, and leaf-surface inoculated	Number of plants inoculated	Number of leaves inoculated	Number of leaves infected	Number of lesions appearing
Pycnospores from living raspberry leaf	Raspberry				
	Under	4	30	21	5,455
	Upper	2	15	0	0
Sphaerulina ascospores from overwintered raspberry leaf	Raspberry				
	Under	1	9	9	387
	Upper	1	8	2	5[a]

[a] These infections appeared near the margin of the leaves and may have been due to faulty technique, or the fungus gained entrance through injuries.

GERMINATION OF PYCNOSPORES, TEMPERATURE RELATIONS, AND CULTURAL CHARACTERS OF THE RASPBERRY FUNGUS

Pycnospores of *Sphaerulina rubi* germinated readily in tap water. In this medium hyphae grew from the pycnospores both terminally and laterally, but most commonly the germ tubes originated from the ends. Evidences of germination, such as swelling and slight elongation, were manifested soon after the pycnospores were placed in water or on nutrient agar. The segments between the septa showed the greatest enlargement and this accentuated the visibility of the crosswalls.

Although this raspberry fungus grew under artificial conditions through a wide range of temperatures, it made its optimum growth at 27° C. Pycnospores germinated in water at 15° C. and produced hyphae 4 or 5 times their length within 72 hours. During the same interval, when held at 27° C. the hyphae attained a length of 10 to 15 times that of the pycnospores.

Isolations of the fungus, obtained from collections made in different parts of the country, showed considerable variation in size of colonies, color of mycelium, and amount of conidial production when grown on corn-meal

nutrient agar. The growth rate of isolates was comparatively slow, some requiring 4 to 6 weeks to cover the surface of an ordinary slant culture maintained at room temperature, while others never attained a diameter greater than 12 to 15 mm. Aerial hyphae varied in amount from sparse to dense, and in color from mouse-grey to white. Color of the submerged mycelium of different isolates varied from white to olive-black, with the olive-black predominating.

In cultures the fungus never produced pycnidia or other forms of functioning fruiting bodies. However, some isolates formed black, spherical bodies on the surface of the medium which resembled fruiting structures but when crushed between a coverglass and slide yielded what seemed to be an oily substance. On nutrient agars the fungus produced conidia profusely, directly from hyphae. Short sterigmata or conidiophores formed along the hyphae, from which several conidia developed successively (Fig. 1, E). Sometimes hyphae formed rings or hyphal coils and sporulated in a manner similar to that described by Kienholz (11) for *Neofabraea malicorticis* and *Glocosporium perennans*. Then too, several anastomosing hyphae may form a strand or rope and sporulate abundantly. In older cultures the hyphae at or near the surface became closely septate, cell fusions were frequent, the whole surface became sporogenous, and produced a great quantity of conidia, piled up in mounds or spread out over the surface as a slimy salmon-colored to fawn-colored layer (Fig. 1, F).

A simple method for observing the manner of early conidial formation was to plant either pycnospores or conidia on a sterile coverglass, cover with a drop of clear nutrient agar, and invert over a Van Tieghem cell. Such a culture, incubated at room temperature, would yield conidia in less than a week. These occupied a narrow plane between the agar and the coverglass, and could be readily examined at the desired magnification.

DISCUSSION

The discovery that a *Sphaerulina* rather than a *Mycosphaerella* is the ascogenous stage of the raspberry leaf-spot fungus, which for many years has been considered to be *Septoria rubi* West., offers an opportunity for speculation as to the probable relationship of this fungus with the forms occurring in dewberry and blackberry.

During these investigations the writers did not find an ascogenous stage in blackberry or dewberry material that could be associated with *Septoria rubi*. The *Sphaerulina*, however, has been plentiful in raspberry leaves each year in Maryland.

The fungus observed on raspberry has not been induced to infect either blackberry or dewberry. Cultures made from blackberry infected the dewberry moderately and the raspberry with difficulty. The fungus isolated from the Lucretia dewberry did not infect the Taylor raspberry, but was moderately pathogenic to the Lawton blackberry. In morphological characters and growth on culture media the raspberry fungus differed from the

forms on blackberry and dewberry, the differences being greater in the case of the Lucretia dewberry form than in the case of the blackberry form. When a particular form infected another host species its morphological and cultural characters were not altered. The variations exhibited by the fungi studied, in the opinion of the writers, indicate that more than one fungus, or else widely divergent strains of the same fungus, are pathogenic for *Rubus* species. Other investigators have observed similar variations. Roark (15) stated that the "blackberry strain would not cross to raspberry nor would raspberry strains infect blackberry." He further stated, in discussing the cultural characteristics of Septoria from *Rubus septoria:* "Strains from the same or different hosts varied considerably . . . some readily forming pycnidia, while others formed only masses of needle-shape secondary conidia." Zeller (21) stated: "Varieties of *R. occidentalis*, *R. strigosus*, and *R. idaeus* are for the most part extremely susceptible to *S. brevispora* and resistant to *S. rubi*, while blackberry species are rather generally attacked by both." Beach (2) inoculated leaves of the black raspberry (*Rubus occidentalis*) and of a species of blackberry with pycnospores taken from the former, and reported: "In 2 weeks all 30 leaves of *R. occidentalis* were thickly spotted with Septoria, but no trace of infection could be found upon the blackberry." He concluded that the results of his experiment "indicated the existence of biologic forms in *Septoria rubi.*"

It is the conclusion of the writers that the raspberry *Sphaerulina* infects neither the blackberry nor the dewberry under natural conditions, and furthermore, the blackberry and dewberry forms probably never infect the raspberry; but it is possible for the forms on blackberry and dewberry to cross-infect under field conditions.

Until there is more substantial proof of the relationship of the leaf-spot fungi from raspberry, blackberry, and dewberry, it seems advisable at the present time to name the raspberry pathogen *Sphaerulina* ***rubi*** n. sp. and retain the binomial *Septoria rubi* West. (*Mycosphaerella rubi* (West.) Roark) to designate the fungus causing the common leaf spot in the *Eubatus* section of *Rubus*.

SUMMARY

A disease of the raspberry known as *Septoria* leaf spot is common in the United States east of the Rocky Mountains and does serious damage in the southern half of that region. A similar disease is common on the blackberry and dewberry throughout this country.

The pathogen in all 3 hosts has been known for almost a century as *Septoria rubi* Westendorp, and more recently as *Mycosphaerella rubi* (West.) Roark.

The present investigation shows that the ascogenous stage of the raspberry fungus is a *Sphaerulina*, while no ascogenous stage of the blackberry and dewberry pathogen could be found in overwintered blackberry and dewberry leaves.

In artificial inoculations under greenhouse conditions the raspberry leaf-spot fungus was non-pathogenic for blackberry and dewberry leaves. The isolates from dewberry leaves infected dewberry and blackberry but not raspberry. Isolates from blackberry leaves infected blackberry readily, dewberry moderately, and raspberry with difficulty.

The isolate—whether to be regarded as a species, race, or strain—from each of the 3 hosts differs morphologically and physiologically from the other 2 and retains its morphological identity even if capable of infecting another host.

The differences are greatest between the raspberry and dewberry forms; the blackberry fungus is intermediate between the other 2 forms.

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COMPARATIVE TOXIC EFFECTS OF EXTRACTS FROM MILD AND VIRULENT ISOLATES OF TOMATO-WILT FUSARIUM

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In the course of studies relating to control of the fusarium (*Fusarium bulbigenum* var. *lycopersici* (Brushi) Wr. and R.) wilt of tomato (*Lycopersicon esculentum* Mill.), work has been published that has dealt with numerous investigations on the action of the pathogen. It has been found that isolates of the fungus differ greatly in pathogenicity (16); that dissimilarity in cultural characters or types are correlated with variations in pathogenicity (15); that this variation is correlated with contrasts in physiology (11); that the virulence of the organism may be increased through selective action, that is, development of variants of exceptional vigor (12, 16); and that the populations of virulent types may multiply and become dominant over mild types under certain environmental conditions, as well as in the presence of tolerant host plants (12). The purpose of the research here reported[2] was to investigate the effects of toxic excretory products of virulent and mild isolates, or "strains," of the tomato-wilt *Fusarium*. A number of workers (2, 4, 5, 6, 7, 17) already have shown that toxic excretory products are formed in cultures of the organism, and a recent report (3) indicates such toxic substances are formed inside the parasitized host cells, as well as in artificial culture. Haymaker (4) compared the relative injurious effects of the toxic substances of a highly pathogenic, with those of a mild, strain of the *Fusarium*, and concluded there were evident differences in effects of the secretions from these two divergent strains. Since my studies dealt with virulent and mild strains of the pathogen similar to those described by Haymaker, it was thought advisable to investigate further any differences in effects of their secretions. No attempt was made to obtain any information on the nature of the substances in secretions from the tomato *Fusarium* that cause the toxic effects.

MATERIALS AND METHODS

In this investigation it was necessary to use a special technique in connection with demonstrating toxic differences. All cultures were grown on agar prepared in accordance with Wellman's formula (12), and the liquids employed were either of this same composition, except that no agar was added, or of the Tochinai composition (9).

Fusarium cultures were grown as follows: Agar Petri plates (9 cm. diameter, containing 25 cc. of medium) were inoculated in the center with

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[2] I wish to acknowledge the valuable assistance of Wilson Levering Smith, Jr. in the studies reported in this paper.

a disk of inoculum and incubated 3 weeks. From around the edges of these plates were cut "standard inoculum-disks," measuring 6 mm. in diameter. A single disk was dropped in an Erlenmeyer flask of 250 cc. capacity, containing 100 cc. of liquid. The inoculated flasks of liquid were then thoroughly agitated, and were incubated in the dark at near 28° C., without further disturbance.

An experiment consisted of comparative tests of a number of series of cultures, each series of different age. The cultures were filtered through No. 2 Whatman filter paper on a Büchner funnel, using a vacuum of 25 inches of mercury. The mats were discarded, except when weighed, and for every different series from 3 to 7 flasks of filtrates of a given age and of a given strain were combined for testing purposes.

In reporting toxicity results each datum represents the arithmetical mean (standard errors were low, from ± 0.00 to ± 0.79, largely around ± 0.06) of observations on from 5 to 18, usually 8, treated plant tops.

The two isolates of *Fusarium bulbigenum* var. *lycopersici* involved in these studies have been described in other publications (11, 12). They differed markedly in cultural appearance and in pathogenic reaction (11). The one (11) described as R5–6 has here been designated as "V" to indicate its relatively high virulence under the experimental conditions. It caused death of susceptible Bonny Best tomato seedlings within 5 to 7 days after inoculation, and killed tolerant Marglobe seedlings in 20 to 24 days. On Wellman's agar this V strain grew slowly, developing a white, powdery, fine, raised and woolly colony. It produced no macrospores but abundant typical unicellular microspores and chlamydospores (18), and had a sweetish aldehyde odor that became ammoniacal and finally disappeared with age. The other isolate, previously described (11) as A15–8, was designated as "M" to indicate its relatively mild infectiousness. It did not seriously injure Bonny Best seedlings, although it produced some wilting of cotyledonary leaves and occasionally affected one or two leaflets on a basal leaf. It caused no observable wilt of Marglobe. On agar it grew more rapidly than V, and developed a dark colored, slimy colony with a few moist, coarse, twisted and tangled "threads" over its surface. It produced large numbers of typical 4-cell, crescent-shape macrospores, unicellular microspores, and chlamydospores (18), and had a yeasty to sourish odor that became ammoniacal and finally disappeared with age. The Bonny Best and Marglobe strains of tomato used for testing were the same as those employed in previous work (15, 16). Comparative effects on these tomato varieties showed that toxic symptoms, *per se*, did not differ although Bonny Best showed a little greater severity in effect in parallel tests.

Dependable toxicity reactions on host plants were successfully obtained when solutions were tested on excised tops from month-old seedlings grown in a standard manner (13) and selected for uniformity. These tops were cut off in the greenhouse while turgid, dropped in water, and carried to the

laboratory where the cut end of each plant was immersed in 30 cc. of test solution in a test tube of 16 mm. by 150 mm. inside dimensions. It was found that nondiluted filtrates from cultures often were so toxic as to cause very rapid plant collapse. When plant tops wilted or collapsed too quickly it was obviously impossible to secure more critical distinctions between comparative toxic disturbances. Trials showed that a good dilution for testing was 1 part of filtrate to 2 of ordinary tap water. Also, it was found that it was neither necessary to filter cultures aseptically nor to sterilize test solutions by filtration through bacteria-excluding filter candles. Care was exercised by washing procedures to avoid contamination between filtrates for test series. For comparative purposes in each experiment, plain tap water was used as one control and another was made from a dilution of liquid medium that had been inoculated in the standard manner but filtered immediately after agitation with the standard inoculum-disk. Spore suspensions to use as a control series to test for toxic effect were prepared also in several instances by gently washing off spores from the agar surfaces of Petri-plate cultures 3 to 6 weeks old.

It has been demonstrated by many workers (1, 2, 4, 5, 6, 7, 17, 19) that excised tops of tomato seedlings wilt when treated with culture extracts not only from the common tomato-wilt *Fusarium*, but from other fusaria, as well, and indeed from fungi of unrelated genera. These results have been further substantiated, as I have found such wilting caused by *Fusarium bulbigenum* var. *lycopersici* (all of the 5 different types (15) of isolates), *F. bulbigenum* var. *niveum* (E. F. Sm.) Wr., *F. oxysporum* var. *cubense* (E. F. Sm.) Wr. & R., *F. oxysporum* var. *aurantiacum* (Lk.) Wr., *F. oxysporum* Schl. f. 2 Wr. (*F. hyperoxysporum* Wr.), *F. sp.* (causing tomato wilt (14)), *Alternaria solani* (E. and M.) J. and G. (several isolates of different types), *Alternaria tomato* (Cooke) Brinkm., *Alternaria sp.* (small-spored, catenulate type), *Penicillium roqueforti* Thom (Strain #160–18 from Charles Thom), and *Aspergillus niger* van Tieghem. It is thus evident that no specificity for wilting of excised tomato tops can be claimed for *F. bulbigenum* var. *lycopersici*. It has been noted, however, that there are different degrees of injury from toxic liquids from this latter organism and that the variations are correlated with dilution, as well as other factors.

All toxicity tests were made in the laboratory at from 26 to 31° C., and were well protected from sunlight or direct air currents. It seemed that the most conclusive wilting developed in the laboratory when the time selected for testing toxic action occurred during a settled period of dry weather.

Haymaker's (4) indices of toxicity as exhibited on plant tops treated with toxic solutions, were described by him as follows: "stem-drooping = 2; leaf collapse = 6; leaves water-soaked = 8; leaves dry and brittle = 10." After some trials it appeared that under my experimental conditions, a different progression of symptoms occurred. It started with slight darkening around the hydathodes at the apices of serrations on the leaf margins, and

advanced by regular steps through varying necrotic and wilting symptoms on leaves to their shrivelling and desiccation. Stem drooping followed by wilt and collapse was considered as a symptom following occurrence of leaf effects. The progression of symptoms was noted in detail in many experiments and the stages of response were finally listed, arranged in order of severity, and given numerical values (Table 1) that were somewhat com-

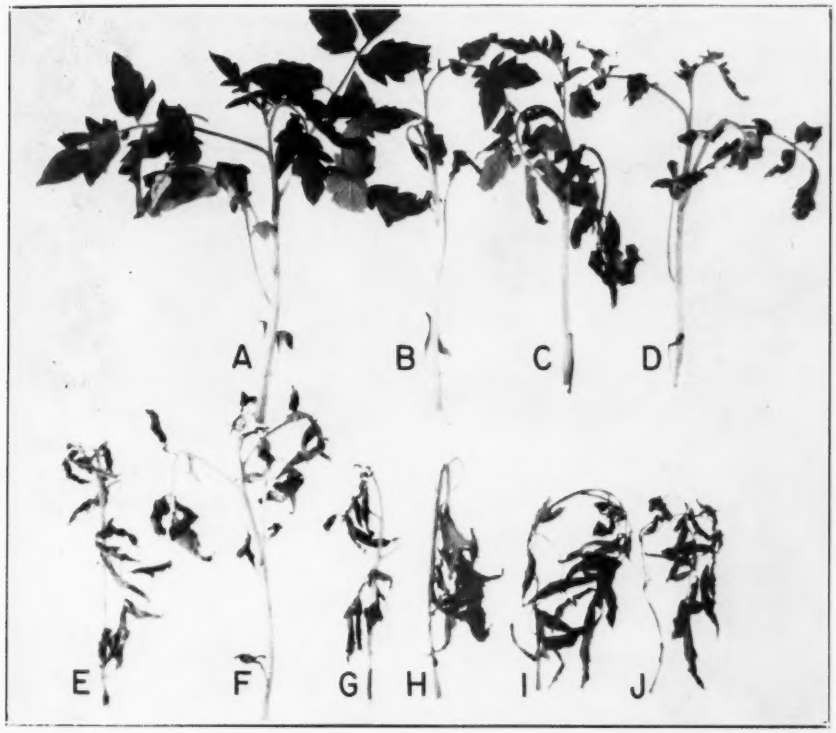

FIG. 1. Different symptoms of toxic effect on excised tops of Bonny Best tomato plants, caused by toxins in filtrates from cultures of tomato-wilt *Fusarium*. A. Included for comparison. No injury, stem immersed in water. B, C, and D. Tests of filtrates taken from mild *Fusarium* isolate during period of its most vigorous growth. E, F, and G. Tests of filtrates taken from virulent isolate during period of its most vigorous growth. H. Test of filtrate from virulent isolate after mat began to show signs of degeneration. I and J. Tests from mild and virulent strains, respectively, during the period in which the mats showed most marked and rapidly changing appearance of degeneration from "staling." Note that E, F, and G appear more seriously injured than B, C, and D. I and J, however, exhibit equally severe collapse. Epinastic effects (see footnote 2, table 1) still observable in C, D, and I. However, when toxic effects were excessively rapid, ending in such severe collapse as in H, I, and J, wilting was seen but epinasty did not occur.

parable to similar values used (9) in estimating pathogenicities of isolates of the tomato-wilt *Fusarium*. The toxic values may be grouped as follows: 0 = no observable injury; 1, 2, 3 = mild injury; 4, 5, 6 = moderately serious; 7, 8, 9 = very serious; 10, 11, 12 = severe; 13, 14 = very severe; and 15 = death.

The most dependable data were from the evaluations taken at 17 to 21 hours after the cut ends of plants were immersed in the test solutions. In

making toxic evaluations, final data were secured by successive recording of evaluations from each experiment at intervals of a few hours during 12 to 48 hours after the tests had been started. At the earliest periods of examination the plants did not appear to exhibit full toxic effects, and after about 26 hours the responses were likely to be irregular and unduly accentuated.

DIFFERENT TYPES OF TOXIC SYMPTOMS

In these studies there appeared to be two general types of symptoms (Fig. 1) : those confined largely to buds, leaflets, and petioles; and those that

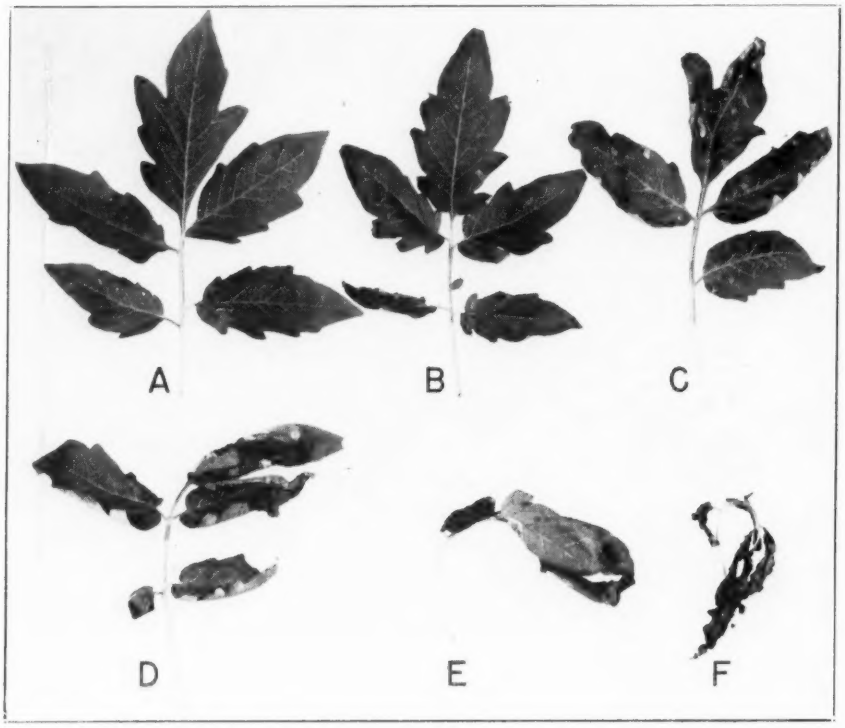

FIG. 2. Symptoms on leaflets of tomato (Bonny Best) of various stages in severity of toxic effect caused by treatment with filtrates of the virulent strain of the tomato-wilt *Fusarium*. For toxic values compare with table 1. Excised plant tops were put in solutions of filtrates from liquid cultures of various ages. A. Included for comparison. No injury, plant top in water, toxic value 0. B. Early symptoms of leaflet injury, plant with toxic value 2. Serrations damaged around the margin of the leaf blades, and one or two small necrotic spots are present in lamina of each leaflet. C. Moderately serious injury, plant with toxic value 4. Increased severity of injury around leaf edges, slight cupping of, and numerous necrotic spots in leaf lamina. D. Serious injury, plant with toxic value 6. Collapse of edges of cupped leaflets, and epinasty. E. Very serious injury, plant with toxic value 8. Wilt of leaflets and epinastic petiole starting to wilt. F. severe injury, plant with toxic value 10. Leaflets crisp-dry and petiole wilted.

consisted of a rather precipitate wilting and collapse of the stem and the whole plant top. These observations were made under the most ideal conditions of experimentation, as outlined above under "Materials and Meth-

TABLE 1.—*Toxic symptoms given numerical values (toxicity indices) and arranged in order of progressive severity of damage. Tests made on excised tops of tomato seedlings*[a] *in solutions from culture filtrates of the tomato-wilt Fusarium*

Toxicity index	Injury on foliage		Injury on stem	
	Leaf blades	Petioles	Apex and bud	Main stem
0	No injury	No injury	No injury	No injury
1	Serrations necrotic on margins of lower leaves	do.	do.	do.
2	Serrations necrotic on margins of lower and middle leaves, with few small necrotic spots in lamina	do.	do.	do.
3	Serrations necrotic on margins of all expanded leaves accompanied by slight cupping, many necrotic spots in lamina	First signs of epinasty,[b] small deflection	Slight injury to serrations on bud leaves	do.
4	Leaves mildly cupped, necrotic spots on laminae, large basal leaflets wilting	Moderate epinastic deflection	Injury more extensive on bud leaves	do.
5	Extensive areas of collapse on leaves, wilting more extensive	Serious epinasty	Severe injury	do.
6	Wilting accompanied by severe cupping, edges collapsed	Severe epinasty, some petioles losing turgidity	Injury on bud leaves more extensive, tips flaccid	do.
7	Severe wilt	First wilt	Tips of bud leaves collapsing	do.
8	Severe wilt, edges starting to dry	All older petioles wilted	All of bud leaves necrotic	do.
9	Leaf edges crinkled and dry	Wilting with collapse	Tip bud and leaves crisped	do.
10	.....	Collapsed	Tip dead but erect	do.
11	.....	.....	Tip of stem wilted	Slight droop of top
12	.....	.....	Crisp dry	⅓ of top collapsed
13	.....	.....	.....	½ of top collapsed
14	.....	.....	.....	All wilted
15	.....	.....	.....	Complete collapse

[a] Seedlings used were of both Bonny Best and Marglobe varieties, grown according to previously described method (13), 4 to 5 weeks old, about 5-leaf stage, in a warm greenhouse, in 3-inch pots. Tops cut off at ground line below cotyledons, dropped in water and then cut ends immersed in test solutions.

[b] Epinasty, as has already been pointed out (10), is typical of an early wilt symptom, and in these studies was characterized by downward deflection of turgid and unnaturally stiffened petioles, in contradistinction to the droop and flaccidity of ordinary wilting.

ods." It was requisite that the respective ages of the cultures tested be of such spacing and continuity that the progressive changes in toxicity of filtrates could be readily followed.

While there was overlapping in toxic symptoms the stages in change were sufficiently distinct to permit their arrangement in progressive order (Table 1). It was repeatedly noticed that symptoms as indicated for toxic

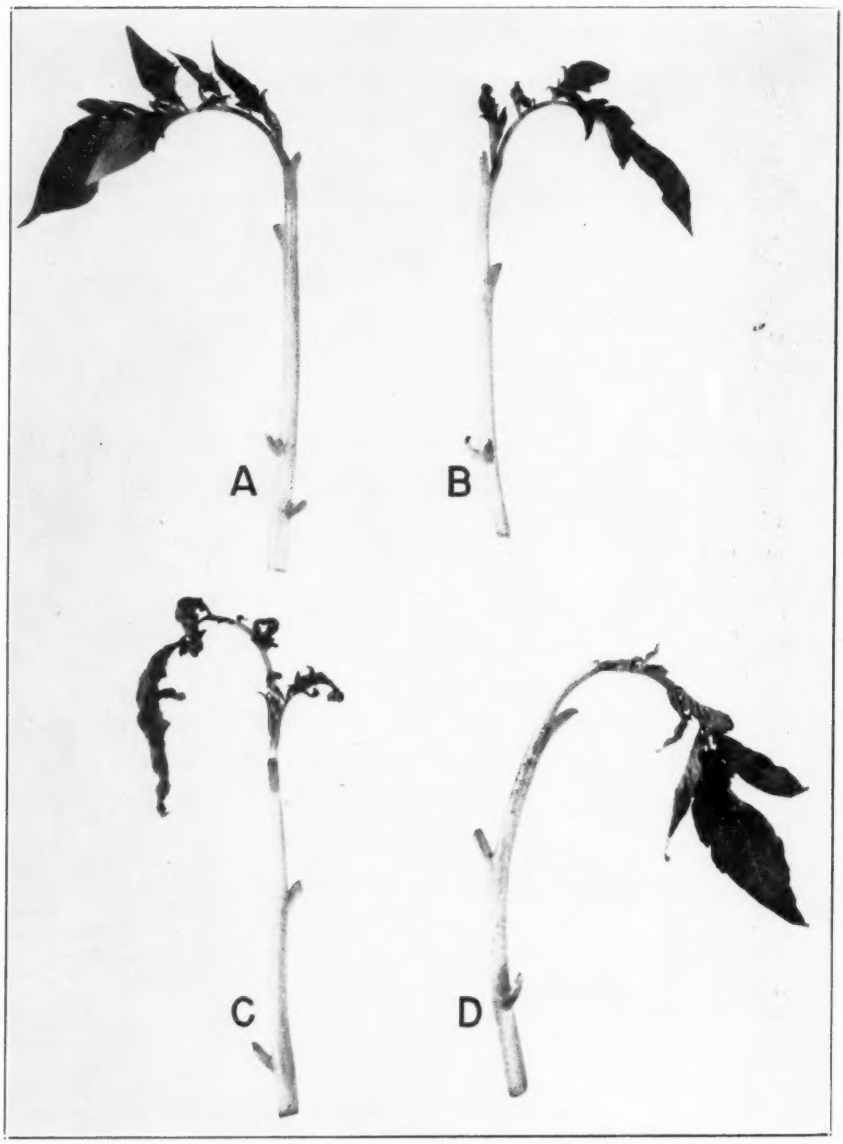

FIG. 3. Symptoms of toxic injury on stem tips and terminal buds of excised Bonny Best tomato plant tops treated with filtrates of growing cultures of wilt *Fusarium* (*cf.* Table 1). A. Included for comparison. No injury, plant in water, toxic value 0. B. Moderately serious injury, effect on edges of youngest tip leaflets, toxic value 5. C. Serious injury, bud and leaflets necrotic, toxic value 9. D. Very serious injury, leaves collapsed, but wilting and stem apex drooping. In another two hours all leaves and bud would have been crisp-dry.

values from 1 to about 10, were developed in filtrates from fresh, well-grown cultures, and symptoms that indicated toxic values from a little above 10 to 15 were from filtrates of cultures that had passed from the stage of vigorous growth to a slightly staled condition. Observations on toxic symptoms

that occurred on plant tops about 18 hours after their cut ends had been immersed in the test filtrates, eliminated somewhat the complications due to excessive drying of toxin-damaged plant parts, that might easily conceal the difference between the collapse from extreme and rapid toxic action that ends in drying, and the slower accumulation of injurious effects that might also end in a desiccated appearance.

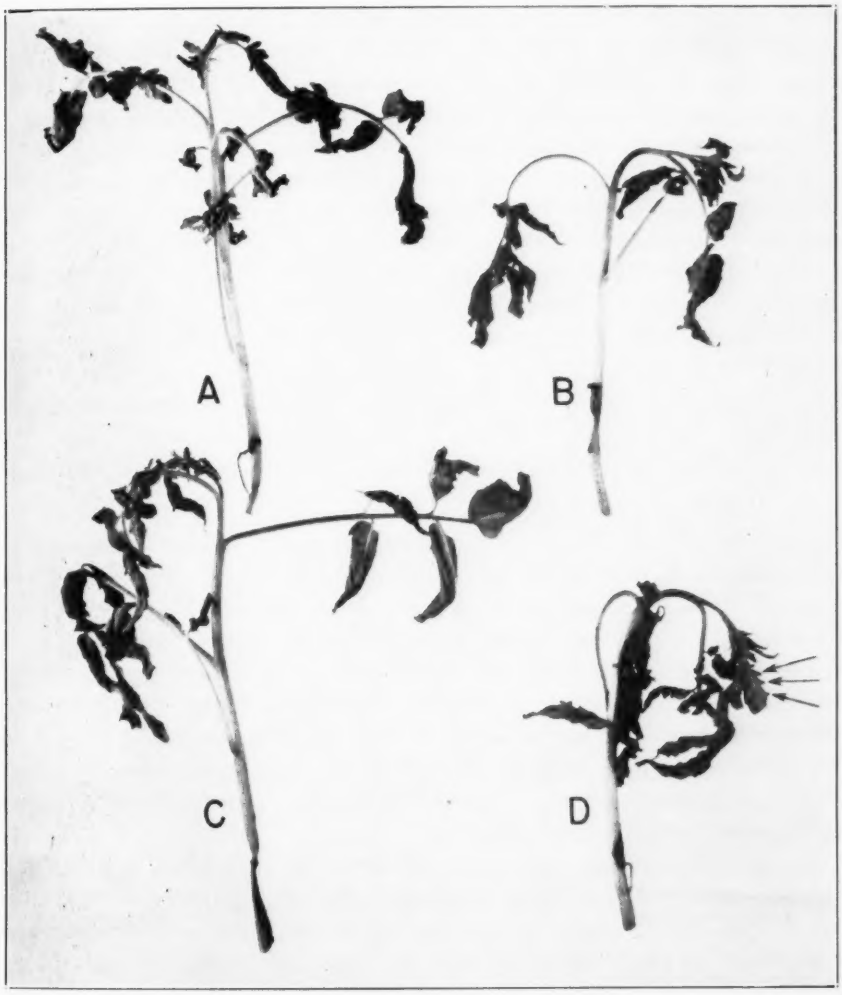

FIG. 4. Comparison of symptoms of different types of toxicity (*cf.* Fig. 1), on excised tips of Marglobe tomato plants, produced by cultures of the wilt *Fusarium*. A and C. Severe injury by filtrate of vigorously growing mats. Shriveled leaflets not quite crisp-dry, epinasty of petioles to be followed shortly by sagging and wilt. B. and D. Wilting response just prior to collapse, due to filtrates from cultures in which mats were showing macroscopic signs of degeneration. Wilting of petioles was not preceded by epinasty. In D, leaflets of the upper leaves were succulent and still showed darkening (indicated by arrows) at hydothodes on serrations around leaf margin. This combination of symptoms occurred rarely in cases of incipient and rapid stem collapse.

It is worth emphasizing that the results of toxic effect from relatively young cultures consisted of symptoms that were almost wholly confined to the leaves. Such symptoms (Fig. 2) were characteristic of plant tests made on filtrates from cultures in which the fungus mats were still in an active state of growth. The symptoms were different in tests from somewhat older cultures. However, it was of interest that, later on, after quite a number of weks of incubation and extreme degeneration of the fungus mats, such leaf symptoms without stem effects were again observable. While these symptoms were of the same general type as those shown in figure 2, they differed from them in that the plants were apt to be a little more flaccid without actually wilting, and the leaves have a slightly grayish cast.

The toxic effects on stems seemed to be of diagnostic value in relation to the condition of the culture from which the filtrates were obtained. Marked stem wilting and collapse as in figures 1, 3, and 4, appeared from treating plants with filtrates from cultures in which the fungus was no longer growing but had recently become "stale" and was in the process of apparent degeneration. In this connection it is well to note that under certain conditions stem collapse occurred so rapidly that leaflets might not be seriously necrosed before wilting (Fig. 4) even though parallel tests with filtrates from younger, vigorously growing cultures might cause extremely severe leaf injury.

COMPARISONS FROM CULTURE FILTRATES

The object of these studies was to compare differences in toxicity of the filtrates between the V isolate that was extremely pathogenic on tomato plants and the M isolate that was very mild in its effect. Preliminary experiments determined the methods to be employed, and assays were made to record in a numerical manner the varying degrees of toxic injury. A total of 10 experiments were conducted in which tests were made on about 2000 excised plant tops. It is from 8 of these tests that the representative data were secured that are presented in table 2, and from which the graphs were drawn (Fig. 5). Findings from 2 of the experiments were corroborative in nature, but were not included in these data because of the relatively small number of series involved.

As has already been mentioned, it appeared that when a sufficiently planned, extensive experiment was conducted and filtrates were studied from cultures of the proper ages, a regular succession of toxic effects would be observed, as in figure 5 and table 2. The tests of media immediately after inoculation, in which no fusarial growth had occurred, always caused greater toxic effects than were seen on plant tops in tap water or in spore suspensions. The beginning of growth of *Fusarium* actually reduced the toxicity of the liquid in which the spores had germinated and hyphae were in an early stage of development (Fig. 5). This beginning period of mild toxicity lasted for about the first week of incubation in V cultures, and more than twice that long in M cultures. In the V cultures there occurred

during about the 6th to the 12th days of incubation, a considerable increase in toxic effect from the culture filtrates, while in the M cultures this occurred from about the 11th to somewhat past the 30th day.

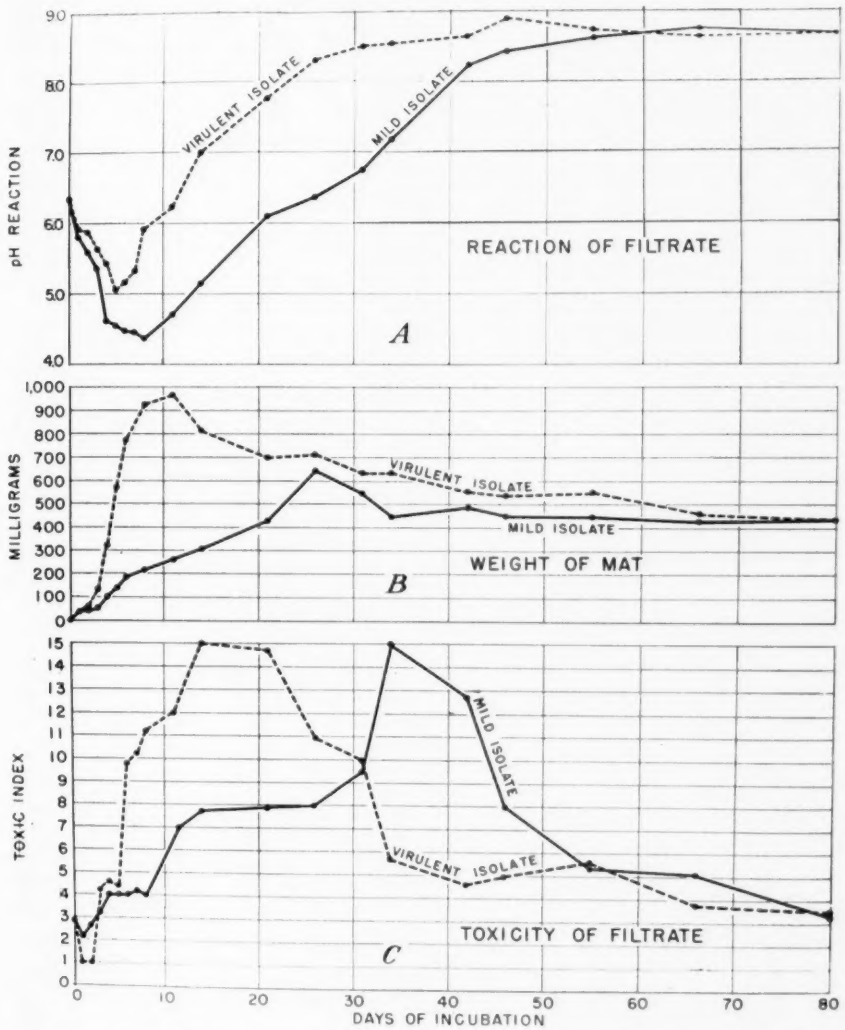

FIG. 5. Results from one typical experiment to compare differences in toxic effect (*cf.* Table 1) of filtrates from cultures at different ages of a virulent and a mild strain of the tomato-wilt *Fusarium*. A. Each pH value is the mean of 3 determinations for each of 3 flasks. B. Each weight is the mean for 3 air-dry fungus mats. C. Each toxicity index is the mean of evaluations of 10 excised Bonny Best tomato plant tops with cut ends immersed in filtrate solutions. Data for A and B secured by methods previously described (11).

It may be seen further (Fig. 5, compare with Table 2) that this period of higher toxicity in both V and M cultures was correlated with the most rapid growth in flasks, as indicated both by increase in mat weight and

TABLE 2.—*Comparison of toxic effects from filtrates of cultures of Fusarium bulbigenum var. lycopersici, from virulent (V) and mild (M) isolates tested at different periods of development*

Experiment No.[d]	Test plant	Isolate	Toxicity of filtrates when mycelial growth of mats was in condition indicated					
			Optimum vigor[a]		First staling[b]		Late degeneration[c]	
			Toxicities[e]	Age of cultures	Toxicities	Age of cultures	Toxicities	Age of cultures
				Days		*Days*		*Days*
1	BB[f]	V	10.0–11.0	7th to 11th	15.0–14.8	13th to 21st	.....	.....
		M	7.2–8.0	13th to 31st	12.8–10.1	35th to 42nd	.....	.....
2	BB	V	9.2–12.0	5th to 9th	15.0–15.0	14th to 26th	4.7	80th
		M	8.3–8.3	14th to 26th	15.0–12.3	34th to 42nd	4.3	80th
	Mg[g]	V	8.7–8.8	5th to 9th	13.7–13.7	14th to 21st	3.2	80th
		M	6.2–6.4	14th to 26th	14.2–12.1	34th to 42nd	3.5	80th
4	BB	V	10.5–10.0	6th to 10th	13.0–12.0	18th to 34th	2.0	95th
		M	5.2–4.7	14th to 31st	14.7–10.4	34th to 55th	2.4	95th
	Mg	V	9.4–8.5	6th to 11th	14.7–9.8	21st to 31st	.....	.....
		M	5.0–4.5	21st to 31st	15.0–12.5	34th to 55th	.....	.....
5	BB	V	9.0–8.0	5th to 11th	14.2–14.1	14th to 34th	2.2	328th
		M	4.0–4.0	16th to 27th	11.0–11.0	31st to 46th	2.3	328th
	Mg	V	.....	.....	10.0–10.0	21st to 31st	1.7	328th
		M	.....	.....	9.4–9.0	33rd to 46th	1.4	328th
8	BB	V	10.4–8.2	6th to 10th	13.0–12.0	14th to 21st	1.6–0.8	115th and 330th
		M	8.8–7.2	16th to 30th	12.1–10.8	38th to 50th	2.7–2.3	115th and 330th
9	BB	V	5.0–5.0	6th to 11th	12.0–11.1	14th to 27th	.....	.....
		M	4.0–3.0	18th to 27th	12.3–7.6	34th to 42nd	.....	.....
	Mg	V	6.0–6.0	8th to 11th	12.0–8.9	14th to 21st	3.5–2.8	100th and 202nd
		M	4.0–3.8	21st to 27th	9.1–7.0	34th to 42nd	3.5–4.3	100th and 202nd
10	BB	V	8.0–7.5	5th to 12th	11.3–9.0	16th to 20th	6.0	412th
		M	5.4–4.6	16th to 27th	.....	.....	6.0	412th

[a] Mat turgid, floating, adhering firmly to inside walls of flask, surface of liquid solidly filled with growth of cheesy to brittle consisteney and of bright colored glistening appearance.

[b] Mats flabby, loosened from attachment to walls of flask, partially submerged, softening, becoming limp, and of dull appearance.

[c] Mats soft, submerged, gelatinous, and of slimy, disorganized appearance.

[d] Data from experiment 3 are presented in graphs shown in figure 5. In experiments 6 and 7, the series were too few and spaced too far apart to give the detailed and proper continuity for the observer to adequately follow the rise and fall in toxicity of successively aged cultures.

[e] Mean values from tests on 5 to 18 plants in each series. Standard errors ranged in most cases from ±0.0 to ±0.36. In experiment 6, due to lack of space, tests were confined to 5 plants for each test-series. These plants were also slightly larger than usual and irregular in growth. Errors for tests in experiment 6 were from ±0.42 to ±0.78.

[f] BB = Bonny Best tomato variety.

[g] Mg = Marglobe tomato variety.

appearance of fungus growth. Following this (Fig. 5, B) mat weights were in a process of considerable reduction, in V cultures about the 13th to the 28th days, and in M cultures from somewhat past the 30th to after the 45th day. I have pointed out before (11) that, after a certain amount of mat growth, autolytic action results in loss of mat weights. It appears from the present data that it is during this period of degeneration that the liquids, recovered from such autolized cultures, caused extreme toxic responses on excised plant tops. The filtrates producing extreme toxic injury were from cultures about two weeks old for the V isolate, and about a month old for the M. The period of extreme toxicity, once started, lasted about 15 days in both the V and M cultures, and that observation correlates well with the time of most rapid reduction in mat weights. As filtrates aged longer they became much less toxic, so that after a few months to a year or more of standing in flasks, the filtrates were much less injurious and of about equally feeble effect whether from V or M cultures.

DISCUSSION

The filtrates from vigorously growing cultures of the highly virulent V isolate of the tomato wilt *Fusarium* were notably more toxic than such filtrates from similar vigorous growing cultures of the mild M isolate. It is possible, therefore, that some qualitative difference could be demonstrated between the toxic substances from the M and V isolates. However, the differences might be only in degree of intensity. Furthermore, when filtrates from cultures of either of the isolates were tested shortly after mats in the respective flasks showed signs of autolysis, the liquids were about equally toxic to tomato plant tops. These assays, it is believed, indicate that the toxic principle most likely to be the actual and precise cause of wilt in fungus-infected plants, can more readily be found in *Fusarium* cultures that are still in a vigorous state of growth. After the fungi stop growing in culture flasks and autolysis sets in, the filtrates obtained from such cultures contain a mixture of decay products and secreted toxins.

Observations reported by others corroborate the evidence of differences in toxic effect of the products from old and young cultures. For example, Hikmet Ahmet (5) found that filtrates from 1-week-old cultures of the tomato-wilt *Fusarium* caused the first wilting of tomato plant tops in 24 hours; cultures that were 2 weeks old produced it in 18–22 hours; those 3 weeks old, in 8–10 hours; those 4 or 5 weeks old, in 5–7 hours; and those 6 or 7 weeks old, in 2–3 hours. He observed also that plants treated for 72 hours in liquids from cultures 1 or 2 weeks old bore wilted and flagged leaves but the stems were erect; if treated in filtrates from cultures 3 weeks old the plants drooped, stem and all; and filtrates from cultures 4 to 7 weeks old caused complete stem collapse. Another example is in the work of Haymaker (4), who used cultures that were 5 and 6 weeks old, and considered a lesser period too short a time to produce the most potent toxin content

[illegible] Mg = Marglobe tomato variety.

in culture. From the data he obtained he concluded, "that in the majority of cases excretory products from virulent strains are apparently no more toxic to plants than those from innocuous strains." However, when he used 3-week-old cultures he found that much greater differences in toxic effect were observable between his virulent and mild strains. It would appear that in most of his experiments his results may have been considerably influenced by a preponderance of autolytic products in his test solutions.

It is, moreover, of practical importance that the mild strain of the *Fusarium* appears much slower than the virulent strain in its toxin-production in liquid culture. It has been pointed out above that these differences in progress in toxicity parallel those in growth of the fungus. In previous work I have reported (12) observations indicating that the internal host environment produces a very distinct inhibiting effect on progress of the mild type of the organism in comparison with the virulent. These observations when taken together may give some clue as to the nature of fusarium resistance in the tomato. As advance of the pathogen in the host tissues is more and more impeded by internal environmental conditions occurring in the tolerant or resistant host types, the infected host has a much better chance to overcome the injury from the parasite. It would appear that effective resistance in the tomato plant may be, at least in considerable part, the result of inhibiting or restraining the growth of the organism once it is within the host rather than of neutralization of a toxin.

Two varieties of tomato were used in these studies, Bonny Best, which is markedly susceptible to fusarium wilt under field conditions, and Marglobe, which is highly tolerant. When comparative reactions from toxic effects of culture filtrates were tested on excised plant tops, it was found that differences in injury were distinct in these two varieties but not so great as would be expected from comparative field data on their relative resistance. It is believed, however, that further understanding of the nature of the wilt toxins and the refinement of the techniques of plant assay involved, might result in development of a method for accurate determination of relative resistance of plants to infection by the tomato-wilt *Fusarium*.

SUMMARY

Studies were made of comparative toxic symptoms appearing on excised tomato plant tops whose cut ends were immersed in filtrates from various aged tomato-wilt *Fusarium* cultures grown in liquid medium. Liquid filtrates from vigorously growing cultures were seriously toxic, causing leaf blade, petiole, and tip bud damage, while filtrates from cultures that had passed the vigorous growth stage and had begun to "stale" caused extreme injury of plant tops including stem collapse. Further aging of liquid cultures appeared to reduce the toxicity of the filtrates to a very marked extent.

Results obtained in comparative assays of a virulent and a mild strain of the *Fusarium* showed that the former strain produced more toxic material

in liquids, and that the filtrates were highly toxic after a much shorter period of incubation than was required by the mild strain. Toxic effects were of about equally extreme severity from staled cultures of either the virulent or the mild strain, and were apparently about equally feeble after long aging of the staled cultures in flasks.

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DIPLODIA PINEA, THE CAUSE OF A DISEASE OF HARD PINES

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INTRODUCTION

Numerous reports and collections of *Diplodia pinea* (Desm.) Kickx (*Sphaeropsis ellisii*[2] Sacc.) on hard pines have indicated its wide distribution in the United States for some time (16, 29, 30, 31), but it is only within the past decade that it has been recognized as the cause of a disease of sufficient importance to arouse a demand by tree owners and commercial arborists for information regarding the disease and its control. The present paper deals with the taxonomy of the fungus, the hosts and distribution, the pathological effects of the fungus by natural infection of various parts of hard pines and by artificial infection of needles and twigs of the current season's growth. The morphology of *D. pinea* has been discussed by various investigators, particularly Curtis (10) and Birch (4); therefore, it is not included in the present study.

TAXONOMY OF THE FUNGUS

Desmazières, in 1842, described *Sphaeria pinea* on dead needles of *Pinus sylvestris* L. in France (12, pp. 104–105). In 1867 the fungus was transferred to the genus *Diplodia* by Kickx (17) and was designated as *D. pinea*, with brown one-septate spores measuring 35–40 $\mu \times$ 16–18 μ. Since then, a similar fungus on various species of pine throughout the world has been reported as a species of *Phoma*, *Macrophoma*, *Sphaeropsis*, *Diplodia*, or *Botryodiplodia*, according to the collectors' individual interpretations of the material at hand. Hence, the synonymy of the fungus has become considerably confused. Birch (4), in his discussion of *Diplodia pinea* on pines in New Zealand, lists 13 synonyms for the fungus, including species from all of the above genera. Stevens, during a study of certain species of *Sphaeropsis*, made spore measurements from authentic material including type specimens, and various collections of a number of these species from pine specimens filed in the herbarium of the Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agricul-

[1] The writer is indebted to Dr. Neil E. Stevens for permission to examine the slides made from exsiccata and collections of *Diplodia pinea* and to make use of his notes in this study; to Mr. Kenneth F. Aldrich, Assistant to Technician, of the New Haven Office, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, for assistance in maintaining the cultures and making inoculations; and to Mr. Bowen S. Crandall of the Division of Forest Pathology for providing transfers of his cultures.

[2] This species name was published by Saccardo as "ellisi" (24, p. 300) and this spelling has been used previously by the writer (29). Freeman Weiss (32, p. 494) stated: "Published by Saccardo as *S. ellisi*, which may have been a typographical error, as it is spelled *ellisii* in the index. The Recommendations governing orthography in the Rules of Nomenclature prescribe the ii form for epithets derived from specific names ending in a consonant (except -er)."

ture, Beltsville, Maryland. In general his results tend to substantiate the synonymy designated by Birch, as indicated particularly by the size of the spores from the following exsiccata:

Diplodia pinea (Desm.) Kickx on dead needles of *Pinus pinea* L. Villa Pamphili, Rome. Mar. 1902. D. Saccardo, Mycotheca Italica No. 955. Spores 27–36 μ × 12–16 μ.

Diplodia pinea (Desm.) Kickx on *Pinus radiata* Don. Natal, South Africa. June 1921. Coll. and det. by A. M. Bottomley. No. 14831. Spores 29.7–36.3 μ × 11.8–15 μ.

Diplodia megalospora Berk. & Curt. on cones of *Pinus taeda* L. South Carolina. 1874. Berkeley Herbarium No. 5012 (type specimen). Also N. A. F. No. 420. Spores 32–39 μ × 12.8–13.2 μ.

Diplodia megalospora Berk. & Curt. on *Pinus nigra* Arnold. Urbana, Illinois. Apr. 7, 1883. Herbarium of A. B. Seymour. Spores 30–36 μ × 12 μ.

Diplodia megalospora Berk. & Curt. on leaves of *Pinus halepensis* Mill. Viscosa, Escola, Brazil. Dec. 8, 1929. Coll. by A. S. Mueller. Det. by N. E. Stevens. Spores 35–41 μ × 16–19 μ.

Sphaeropsis pinastri Cke. and Ell. on *Pinus sylvestris.* Newfield, New Jersey. 1878. Ellis Herbarium No. 2917. Det. by Cooke. (Type specimen.) Saccardo used this specimen as the basis for establishing the species *S. ellisii* (24, p. 300) since he had already given the name *S. pinastri* (24) to a fungus reported by Léveillé in 1846 as *Phoma pinastri* (20, p. 282). A fungus collected by C. J. Muller at Eastbourne, England, on "fir" cones (presumably Scotch pine) was designated as *Phoma pinastri* Lév. by Cooke in 1878 (7, p. 178). No spore measurements were given in Léveillé's description, but Cooke reported the spores in his material as 10 μ × 6–7 μ. Grove (14) stated that Saccardo used Cooke's measurements in his description of *S. pinastri,* but that the spores in Cooke's specimen are identical in size with those reported for *S. ellisii* Sacc. (*S. pinastri* Cke. and Ell.). He concludes, therefore, that *P. pinastri* Lév. is synonymous with *S. ellisii* Sacc. It is possible that Cooke's specimen labeled *P. pinastri* Lév. may be mixed material, and a portion of it may be identical with *Diplodia pinea* (*S. ellisii*). The inclusion of *P. pinastri* Lév. as a synonym of *D. pinea,* however, cannot be made without a detailed examination of authentic material, not available to the writer. A specimen, numbered 4777 in the Fungi Columbiana and labelled "*Sphaeropsis pinastri* (Lév.) Sacc., on cone bracts of *Pinus nigra* London, Ontario, Canada. April 1915. Det. by J. Dearness," has spores measuring 30–36 μ × 12–13.5 μ and apparently should have been labeled *Sphaeropsis pinastri* Cke. and Ell. or *S. ellisii* Sacc.

Sphaeropsis ellisii Sacc. (*S. pinastri* Cke. & Ell.) on fallen cones of *Pinus nigra.* Newfield, New Jersey. Jan. 1894. Coll. J. B. Ellis No. 967. N.A.F. No. 525. Spores 30–36 μ × 12–15 μ.

Sphaeropsis ellisii Sacc. on cones of *Pinus sylvestris.* Lombardy. Cavara.—Fungi longobardiae exsiccata No. 95. Spores 30–39 μ × 12–18 μ.

Sphaeropsis pinicola Speg. on needles and twigs of *Pinus halepensis*. Fort Cunyngham, Cape of Good Hope, South Africa. Jan. 4, 1913. Coll. and det. by I. B. Pole Evans. Sydow's Fungi Exotici Exsiccati No. 279. Spores 33–39 μ × 14–18 μ.

Additional exsiccata, examined by Stevens, with similar spore measurements, which vary noticeably from those given in the original descriptions of the respective species, are as follows:

Diplodia conigena Desm. on cones of *Pinus sylvestris*. Padova, Italy. Jan., 1898. D. Saccardo, Mycotheca Italica No. 355. Spores 28.5–39 μ × 12–15 μ.

Diplodia conigena Desm. on cones of *Pinus pinea*. Villa Pamphili, Rome. Mar., 1902. D. Saccardo, Mycotheca Italica No. 956. Spores 30–33 μ × 12–15 μ.

Diplodia pityophila Fckl. on dead branches of *Pinus sylvestris*. Budenheim, Austria. Herbier Fuckel 1894. Fungi rhenani No. 538. Herbier Barbey-Boissier No. 2299. Spores 30–36 μ × 12–16 μ.

Diplodia sapinea (Fr.) Fckl. on twigs of *Pinus sylvestris*. Padova, Italy. June, 1897. D. Saccardo, Mycotheca Italica No. 160. Spores 28–36 μ × 12–15 μ.

Diplodia sapinea (Fr.) Fckl. on twigs of *Pinus sylvestris*. Norfolk, England. Aug., 1874. Coll. by C. P. Plowright. DeThümen, Mycotheca Universalis No. 383. Spores 30–39 μ × 12–13.5 μ.

Birch does not include these species in his synonymy, but Grove gives *Diplodia conigena* as a synonym for his *D. pinastri*, and *D. sapinea* as similar except for spores "sometimes inequilateral" and measuring 20–26 μ × 12 μ. Haddow and Newman (15) found that *Sphaeropsis pinicola* Speg., originally collected from *P. radiata* in Argentina, is also the same as *D. pinea*. These are only a few examples of the confusion encountered in checking over the exsiccata and collections of this fungus. However, Desmazière's *Sphaeria pinea*, changed to *Diplodia pinea* by Kickx, antedates the other species mentioned and therefore *D. pinea* is the preferred designation.

HOSTS AND DISTRIBUTION

Sphaeria pinea was originally described on dead needles of *Pinus sylvestris* in France (12, pp. 104–105). When it was changed to *Diplodia pinea* its habitat was given as needles of *P. sylvestris* and *P. mugo* var. *mughus* (Scop.) Zenari in Belgium, France, and Italy (17). In the United States *D. megalospora* was described on cones of *P. taeda* in South Carolina (3, p. 3) and a similar fungus, *Sphaeropsis pinastri*, was reported on dead twigs of *P. sylvestris* in New Jersey (8, p. 5). Since that time *D. pinea* has been reported under its various synonyms on several species of pine. Many of the early reports did not indicate definitely whether the fungus was found merely on dead parts or on living leaves and twigs, although in some cases it was mentioned as occurring on dead leaves hanging on the trees or on cones. Since the fruiting bodies usually appear only after the leaves or

twigs have been killed by the fungus, most of the exsiccata and collections were from dead material. From the early records, therefore, it is difficult to determine the role of *D. pinea* as a saprophyte or a parasite.

The earliest known reports of *Diplodia pinea* as the cause of a disease of pines were published from the Royal Botanical Gardens, Kew, England, in connection with the occurrence of infected nursery stock and adult trees of *Pinus radiata* and *P. mugo* var. *mughus* at Cape Colony, South Africa (1, 2). In that study inoculation experiments showed that the fungus isolated from *P. radiata* would infect *P. sylvestris* as a wound parasite. A die-back of trees in exotic pine plantations, due to *D. pinea*, has been under investigation in Australia (21, 34). *Pinus radiata* is the most severely attacked, while less susceptible species include *P. ponderosa* Laws., *P. pinaster* Ait., *P. patula* Schl. & Cham., *P. roxburghii* Sarg., *P. caribaea* Mor., and *P. coulteri* Don. In New Zealand two comprehensive studies of the disease on exotic forest pines, particularly *P. radiata* and *P. muricata* Don, were made by Curtis (10) and Birch (4). The latter found that *P. ponderosa*, *P. nigra poiretiana* (Ant.) Aschers. and Graebn., and *P. contorta* var. *latifolia* S. Wats. also were susceptible.

In Europe various exsiccata and references to collections of *Diplodia pinea* and its synonyms indicate its wide range of distribution, particularly on *Pinus sylvestris*, but its importance as the cause of disease has been mentioned only occasionally. It has been reported as a dangerous parasite on pine in Russia (6), on *P. sylvestris* and *P. nigra* in Rumania (23), and on *P. sylvestris* in Austria (27). Grove (14) stated that *D. pinea* occurs as a serious pest on leaves and young branches of *Pinus* (*sylvestris*, *mugo* var. *mughus*), "south of England, rather common," and that *D. pinastri* Grove, which seems to be synonymous, is rather common in the British Isles on bark, leaves, and cone scales of *P. sylvestris*, but he made no statement concerning the importance of this latter species as a parasite.

In the United States the first mention of *Diplodia pinea* as a parasite, causing cankers and a dying of the needles of pine in New Jersey, was made by Schwarze (25, pp. 86–87) in 1917. Hedgcock (16) reported *D. pinea* or closely related species from 14 States in the eastern and central United States as a weak parasite often following insect or other injuries on 11 species of both hard and soft pine:—*P. echinata* Mill., *P. griffithii* McClell., *P. flexilis* James, *P. muricata*, *P. nigra*, *P. pinea*, *P. radiata*, *P. resinosa* Ait., *P. rigida* Mill., *P. strobus*, and *P. sylvestris*. Several of these collections antedate Schwarze's publication and Hedgcock's notes show that the fungus was recognized as the probable cause of disease on *P. nigra* as early as 1908. Reports of the distribution of the disease as indicated by specimens examined by the writer (29, 30, 31) have mentioned its occurrence from 21 States on *P. nigra*, *P. sylvestris*, *P. mugo* var. *mughus*, *P. ponderosa*, and *P. resinosa*. The disease has been reported by Lancaster (18) from Nebraska on *P. nigra* and *P. sylvestris*, and by Slagg and Wright (26) on 10- to 50-year-old trees of *P. nigra*, *P. ponderosa*, and *P. sylvestris* in Manhattan, Kansas.

Most of these collections have been from ornamental trees, but the fungus has been reported by Crandall (9) on nursery trees of *P. resinosa* causing a collar and root rot and by Slagg and Wright (26) on seedlings of *P. nigra* and *P. ponderosa*, also on 2- and 3-year-old stock of *P. nigra*, *P. ponderosa*, and *P. sylvestris*. These various collections and published reports available to the writer indicate that one or more instances of infection have been found in the following States: Maine, New Hampshire, Massachusetts, Rhode Island, Connecticut, New York, New Jersey, Pennsylvania, Maryland, Delaware, Virginia, West Virginia, North Carolina, South Carolina, Kentucky, Tennessee, Ohio, Illinois, Michigan, Wisconsin, Minnesota, Iowa, Missouri, South Dakota, Nebraska, Kansas, and Oklahoma. It is very probable that the disease is even more widely distributed.

Elsewhere in America only occasional collections and reports of *Diplodia pinea* have been found. In Ontario, Canada, Haddow and Newman (15) reported the fungus as the cause of a tip and twig blight of various conifers, in plantations and in the natural forest, but this type of disease was of relatively slight importance. In association with the pine spittlebug, however, the fungus caused serious injury to twigs, branches, and stems of *Pinus sylvestris*, particularly on trees over fifteen years old. In South America *D. pinea* was described under its synonym *Sphaeropsis pinicola* from fallen needles of *P. radiata* in Argentina (15) and as *D. megalospora* on needles of *P. halepensis* in Brazil. The collections of the fungus from the various countries and the literature citations just mentioned indicate that *D. pinea* is the cause of disease of more or less importance in the Western Hemisphere within the range of 30–50 degrees latitude both north and south, and within the same latitude range in the Eastern Hemisphere.

Of the 11 species of hard pine mentioned as hosts for *Diplodia pinea* in the United States, the number and frequency of collections from *Pinus nigra* suggest its high susceptibility (29). *Pinus sylvestris* and *P. mugo* var. *mughus* also are highly susceptible, and occasional instances of infection on *P. ponderosa* and *P. resinosa* have been noted. *Diplodia pinea* is not known to cause any serious disease of well-established trees of these hosts in forest plantations. Only one case of infection has been reported, so far as known, on each on the other 6 species, all of these, except *P. pinea*, being indigenous to the United States. From this it seems evident that it is the exotic pines (*P. nigra*, *P. sylvestris*, *P. mugo* var. *mughus*) that are most susceptible. Boyce (5) points out the fact that certain exotic coniferous forest trees are frequently severely attacked by fungus diseases that are not serious on the same species of trees in their native range. The sparsity of reports on the disease within the native ranges of the susceptible species of pines would seem to indicate that Boyce's statement may well apply to *D. pinea*. This also corresponds with the reports of the disease in Australia (21) and New Zealand (4), where the exotic species *P. radiata* and *P. muricata* are particularly susceptible. Birch (4) considers *D. pinea* as "almost ubiquitous in exotic pine plantations throughout New Zealand, growing as a saprophyte

in dead bark, wood, needles, cones, and general pinaceous débris" and concludes that it is parasitic "only on trees weakened as a result of unsuitable growing conditions." In the case of ornamental trees of the susceptible species of pine in the United States poor planting methods and unfavorable soil conditions may predispose the trees to infection, but no data on this point are available.

PATHOLOGICAL EFFECT OF DIPLODIA PINEA

Natural Infection

The most pronounced symptom of the disease caused by *Diplodia pinea* on hard pines in the United States is the killing back of the current season's growth year after year until the tree becomes stunted and sometimes eventually killed. This usually is a slow process, since most frequently the lowest branches of old well-established trees are first affected, and the disease spreads upward very gradually. If young trees are attacked the killing takes place much more rapidly. Very few instances are known in which large trees have been killed by the disease. This is due to the fact that before the disease has progressed over the entire tree the repeated killing of young terminals has made the tree unsightly and undesirable as an ornamental, and control measures are employed or the tree is removed.

The fungus may attack the current season's growth in any one of a number of ways: by direct infection of young needles as they develop; by infection of the elongating shoot; by the mycelium present in one bud of a terminal cluster spreading to the adjacent buds or into the twig as growth advances; and by mycelium from a twig infected the previous year causing a slow weak growth that rapidly becomes invaded by the fungus. The first of these is observed particularly on newly infected trees. Infection in such cases takes place at the base of the needles and prevents their further growth as the mycelium spreads through the leaf tissue. A few infected needle fascicles may be scattered among the healthy needles of the young shoot. When the needles are brown and dead the fruiting pustules of the fungus appear at the base of the needles and even on the leaf sheaths. The production of the fruiting bodies progresses outward toward the tip of the needle. If the disease is prevalent in the vicinity and seasonal conditions are favorable for spore germination, more extensive infection may occur. On *Pinus sylvestris* the mycelium resulting from infection of the needles may extend from the needles through the tissue of the leaf scars into the twig, forming cankers around the leaf scars. Fruiting bodies soon appear on the twig, and the cankers increase until the twig is girdled. A slight amount of infection is easily overlooked and for this reason the tree owner usually does not realize that his trees are attacked until continued infection follows in successive seasons. Also the fact that the fruiting bodies do not appear until the needles are dead results in uncertainty in making an early diagnosis of the trouble. On *P. mugo* var. *mughus* infection of needles and buds has been found to result in the formation of slowly developing cankers

at the nodes. A die-back of all twigs above the affected nodes occurs very rapidly. Mycelium spreading through the needles into the twigs grows slowly in *P. nigra*. Its effect is not particularly noticeable until it has progressed down the twig toward the node, girdling the twig and thus prohibiting the further growth of the young shoot. Dead needles with sporulating fruiting bodies may hang on these twigs for two seasons and provide a source of inoculum that will infect the new growth.

It sometimes happens that a few needles adjacent to the bud cluster become infected late in the season, and one of these buds, usually a lateral, may become invaded by the fungus. Externally, the only evidence of invasion is an excessive resinosis on and around the bud. Because of the normal resinous condition of buds of *Pinus nigra*, this resinosis usually escapes detection. Sections through such a bud show partial or complete penetration by mycelium. The bud fails to develop during the following season, but the neighboring buds may begin growth normally, only to succumb when the needles are partially grown. Thus, in a cluster of buds, one may be entirely dead, another may develop into a shoot with stunted brown needles, and a third, usually the terminal bud, may produce a fairly normal shoot with a shortened internode, a majority of mature needles, a few stunted diseased needles, and one terminal bud. It is evident that the following year this bud may develop a weak shoot that will soon become infected. This type of infection results in the production of conspicuous clusters of brown, stunted needles with abundant fruiting bodies and an excessive production of resin in both twigs and needles causing a heavy deposit of resin on the bark and the clinging together of affected needles in resinous masses. These symptoms are the most easily recognized by the tree owner and are considered typical of the disease.

Infection of the succulent tissue of an elongating shoot may take place at any time in its development until the needles are about half-grown. The most usual period, however, is that prior to the emergence of the needles from the leaf sheaths. The needles involved in the affected tissue soon cease growth, turn brown, and die. Other adjacent needles may develop to half size but succumb to the effects of the advancing mycelium. Still other needles, particularly near the base of the same shoot, may attain maturity and retain their normal green color apparently unaffected by the fungus.

Although the disease most commonly results in a tip blight or die-back of the young growth, other parts of susceptible pines may become invaded by the fungus. If a cone-bearing twig is infected, the mycelium has been found to penetrate from the twig into the core of the young cones, which then fail to develop. Fruiting bodies of the fungus are frequently abundant on the apophyses of the cone scales of large mature cones or of fallen cones and also on the wings and seed coats. In some cases the trees on which these cones form show no evidence of infection on twigs or leaves, and the fungus seems to be saprophytic. The writer has observed that dead seeds filled with mycelium may be present occasionally, but in most cases the

fungus is confined to the surface of the seed coats. Apparently it infects poorly developed seeds, but is incapable of infecting normal healthy seeds. There is a possibility, however, that its presence on the seed coats might be a source of infection for young seedlings as they emerge from the ground bearing the seed coats on the young leaves. So far as known to the writer this possibility has never been tested. Slagg and Wright (26) reported the infection of pine seedlings 5 months after the seed was sown, but the source of infection was not determined. Birch (4) reports that tests with seed of *Pinus nigra poiretiana* from Canterbury, New Zealand, and *P. ponderosa* from the northwestern United States showed a very small percentage of infected seed. He believed that the fungus occurs as a saprophyte on the surface of the seed coats and in the interior of dead seed.

A collar and root rot of nursery trees caused by *Diplodia pinea* has been reported by Crandall (9) on 3- to 5-year-old seedlings of *Pinus resinosa* in Maryland. Stem infection by *D. pinea* in 7- to 15-year-old trees of *P. ponderosa* and *P. radiata* in stands in New Zealand (4) caused the death of trees if penetration by the fungus occurred near the ground level. A sap stain in southern pine lumber in the United States was attributed by Davidson (11) to *D. pinea* (*D. megalospora*), and a similar sap stain was described by Verrall (28), as caused by an undescribed species of *Diplodia*, possibly a strain of *D. megalospora*. Staining of the sapwood of *P. pinea* from which *D. pinea* was isolated has been studied in detail by Goidanich (13) in Italy in timber and in living trees weakened by severe root pruning or by borers. In New Zealand also (4) the fungus has been found to produce a staining of timber from *P. contorta* var. *latifolia*, *P. radiata*, *P. ponderosa*, and *P. muricata*, but living trees are not susceptible "unless in advanced stages of disability."

Artificial Infection

In a study of tip blight and die-back of *Pinus nigra*, *P. mugo* var. *mughus*, and *P. resinosa* caused by *Diplodia pinea*, White (33) successfully inoculated the young growing tips of potted trees of *P. mugo* var. *mughus* and *P. sylvestris* in the greenhouse with spores isolated from infected needles of *P. nigra*. Later, Pirone (22) found by inoculation that 3- to 6-year-old trees of *P. mugo* var. *mughus*, *P. sylvestris*, and *P. nigra* were susceptible to stem infection by *D. pinea*, similar to that described by Crandall (9) from inoculations of 3-year-old seedlings of *P. resinosa*. Haddow and Newman (15) reported the successful inoculation of healthy shoots of *P. sylvestris* in the field with a suspension of spores of *D. pinea* from infected pine twigs of the same host. The inoculum was injected an inch or two below the terminal buds of leading and lateral shoots. Of the 30 shoots inoculated, all showed infection after 3 months.

In the present study the effect of *Diplodia pinea* on the current season's growth of 4 species of hard pine and 1 of soft pine was tested by inoculating young nursery trees of various ages (approximately 5–10 years) in an experimental plot, growing under exceptionally favorable conditions. The

species of pine were as follows:—*Pinus nigra* (trees 2′–4′ in height); *P. sylvestris* (2′–6′); *P. resinosa* (2′–4′); *P. ponderosa* (2′–3′); and *P. strobus* (1½′–3′). Single-spore cultures of *D. pinea* isolated from needles of *P. nigra* collected at Hamilton, Mass., Southold, Long Island, N. Y., and Philadelphia, Pa., and grown on Leonian's synthetic medium (19), were the source of inocula. In a few cases a loopful of freshly exuding spores from pycnidia in culture was used as inoculum, but in most cases immature fruiting bodies or bits of mycelium were substituted for the spores, since the fungus did not fruit readily in culture. The kind of inoculum did not seem to influence the results. Inoculations were made in April, and June, 1934, May, 1935, May, 1936, and June, and July, 1937. Because of the fact that the current season's growth did not develop uniformly on all the trees a limited amount of growth was usually available at any one time when inoculations could be made. Therefore, in some cases, only one inoculation of a kind could be made on each host. The results are necessarily inconclusive but give a general indication of the conditions under which infection may take place. Whenever two or more inoculations were made on one tree, particular care was taken so that these should be on widely separated bud clusters or shoots to prevent the possibility of cross infection. The inoculum was applied to the trees in five different ways: 1. On the surface or at the base of uninjured buds early in the spring before new growth started; 1. On buds injured by piercing the bud scales near the base of the bud with a sterilized needle; 3. On the surface of uninjured leaves of the new growth, near the bases of the leaves when they were about 1 inch long; 4. On leaf scars from which the leaves of the new growth had just been removed; 5. In wounds on young twigs, about 1″–3″ above the node, made by cutting with a sterilized scalpel a small triangular section of bark under which the inoculum was inserted. In all cases after the inoculum was applied the portion inoculated was wrapped with moist cotton, over which was firmly tied a strip of heavy waxed paper. The coverings were removed 1 to 2 weeks after inoculation, depending upon the weather conditions and the rate of development of the current season's growth. Controls were made to compare with the different types of inoculations except that no inoculum was introduced.

The results, as shown by table 1, indicate that uninjured buds of *Pinus sylvestris, P. resinosa*, and *P. ponderosa* may become infected. In 3 cases the new growth from these buds developed until the first needles attained a length of about 1 inch. Both leaves and twigs of the new growth remained chlorotic and stunted, soon turning brown. Early in the following spring the fungus was reisolated from two of the brown twigs. The other infected twig, showing fruiting bodies the next spring, was left on the tree to determine whether the fungus would spread farther. In the fall of the same year, however, there was no indication that the fungus had advanced into the previous year's growth. The twig was removed and the fungus was reisolated from the affected tissue. Two of the inoculations made on uninjured buds resulted in the infection of scattered needles, particularly at the

tip near the terminal bud. These were left on the trees until the following spring, when the advance of the fungus into the twigs became noticeable. The one terminal bud on each of these twigs developed only a weak growth and both needles and twigs of this growth became infected, the twigs being killed back to the nodes. In the fall they were collected and the fungus was reisolated. In another case of needle infection only a few scattered needles became infected the first season. These were left on the tree and slight infection of needles appeared in each of the two following seasons. After the second season the fungus was reisolated from infected needles. In the two remaining inoculations reported from uninjured buds, both of

TABLE 1.—*Results of inoculations of species of Pinus with Diplodia pinea isolated from Pinus nigra*

Part inoculated	Species inoculated	Number of	
		Inoculations	Infections
Buds uninjured	*nigra*	1	0
	sylvestris	11	3
	resinosa	9	4
	ponderosa	2	1
Bud scales injured by needle puncture	*nigra*	1	1
	sylvestris	6	3
	ponderosa	1	1
Leaves uninjured	*nigra*	2	1
	sylvestris	3	3
	resinosa	3	2
	ponderosa	1	1
	strobus	1	0
Leaf scars—leaves removed	*nigra*	2	1
	sylvestris	1	1
	resinosa	1	1
	strobus	1	0
Twig wounded by slitting bark	*nigra*	3	3
	sylvestris	3	3
	resinosa	1	1
	strobus	1	0

which were made on leaders, one lateral bud in each inoculated cluster failed to develop, but the terminal bud put out normal healthy growth. Infection in the lateral bud spread into the main twig, and in both cases cankers developed during the following season. These resulted in a girdling of the twig, killing the growth that had been produced by the terminal buds following inoculation. Fruiting bodies of the fungus appeared on the cankers and the organism was reisolated.

When the buds were injured by piercing with a sterilized needle, infection took place on *Pinus nigra, P. sylvestris,* and *P. ponderosa.* Two instances of infected shoots, one of infected scattered needles, and two of infected lateral buds developing into cankers resulted in symptoms similar to those produced from the infected uninjured buds. On *P. nigra* the inoculated lateral bud on the leader became infected and a canker was gradually formed, beyond which the uninfected terminal buds put out new

growth for three seasons. The following season the canker had developed sufficiently to girdle the stem (Fig. 1, A) and the entire top died back. The fungus was reisolated from the canker, as well as from the other four cases of infection from injured buds reported in table 1. In this series of inoculations the controls produced a fairly normal growth, at first slightly

FIG. 1. Cankers resulting from inoculations with *Diplodia pinea*. A. Four-year-old canker from infection of injured bud of *Pinus nigra*. (Approximate × 1½.) B. Canker 3 months after inoculation of twig wound on *P. sylvestris*. (Approximate × 1¼.) Photographs by H. G. Eno.

chlorotic, but apparently the piercing of the bud scales with the needle did not permanently injure the growing tissue.

Positive results were obtained from seven of the inoculations on uninjured leaves. Numerous brown needles with fruiting bodies of the fungus were evident in the autumn following inoculation, and small cankers with fruiting bodies were formed on the twigs around the base of the infected needles. Specimens were collected and the fungus was reisolated. The controls in this series were chlorotic at first, soon becoming normal. When the inoculum was applied directly to the leaf scars from which the leaves had just been removed, infection occurred in 3 instances, causing small cankers and diseased needles. The fungus was reisolated from two of the cankers in the autumn following inoculation and from the third canker the next spring. There was no evidence of injury in the controls.

The fifth series of inoculations indicates that the fungus will readily infect the host through wounds. Definite cankers, with considerable resinosis on the infected needles and twigs (Fig. 1, B), as well as an abundance of fruiting bodies on the cankers and surrounding needles, were conspicuous three months after inoculation. A month later, at the end of the growing season, the fungus was reisolated from 5 of the cankers. The other 2 cankered twigs were left on the trees during two growing seasons and the fungus was reisolated from them in the following fall. All the controls in this series showed normal wound healing.

In June, 1934, mycelium from a culture of *Diplodia pinea,* isolated by Crandall from the stem of a 3-year-old seedling of *Pinus resinosa* showing collar and root rot (9), was used as inoculum in four inoculations as follows: two inoculations on uninjured buds of *P. nigra,* one on an uninjured bud of *P. resinosa,* and one on a lateral bud of the terminal cluster on *P. nigra* injured by piercing with a sterilized needle. Positive results were obtained from the third and fourth inoculations. From the uninjured infected bud the new growth developed only partially, with leaves and twig soon turning brown. In the following spring fruiting bodies were abundant on the twig that was killed back to the node, but the fungus spread no farther. In the fall the fungus was reisolated from the diseased tissue. In the fourth inoculation, infection of the injured lateral bud resulted in the formation of a canker at the node. The fungus did not spread beyond that area, however, and was reisolated in the spring of 1938.

SUMMARY

The disease of hard pines caused by *Diplodia pinea* (Desm.) Kickx (*Sphaeropsis ellisii* Sacc.) is widely distributed in the United States and has also been reported from various countries in both Hemispheres within the latitude range of 30 to 50 degrees both north and south. In the United States the exotic hard pines, *Pinus nigra, P. sylvestris,* and *P. mugo* var. *mughus,* are the most susceptible, but the fungus occurs occasionally on eight other species of hard pine. The disease has not yet been known to cause any

extensive injury in forest plantations but frequently it is of considerable importance on ornamental trees.

The killing back of the current season's growth of ornamental trees for several successive seasons results in the weakening of the trees and sometimes in the death of entire trees. The disease may attack the new growth in any one of a number of ways: by direct infection of young needles; by infection of the elongating shoot; by infection of one bud of a terminal cluster from which the mycelium spreads into adjacent buds or into the twig; and by spread of mycelium from a twig infected the previous year.

Inoculations were made in an experimental plot on young trees of *Pinus nigra, P. sylvestris, P. resinosa, P. ponderosa,* and *P. strobus* using as inoculum mycelium or immature fruiting bodies produced in single-spore cultures of *Diplodia pinea* isolated from infected needles of *P. nigra,* and from stem cankers on *P. resinosa.* Positive results were obtained from the inoculations of uninjured buds of *Pinus sylvestris, P. resinosa,* and *P. ponderosa;* of injured buds of *P. nigra, P. sylvestris,* and *P. ponderosa;* of uninjured leaves of *P. nigra, P. sylvestris, P. resinosa,* and *P. ponderosa;* of leaf scars of *P. nigra, P. sylvestris,* and *P. resinosa;* of twig wounds of *P. nigra, P. sylvestris,* and *P. resinosa.*

The results of the inoculations indicate that *Diplodia pinea* will infect healthy actively growing tissue of buds and leaves but will infect more readily through wounds.

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STUDIES ON THE MORPHOLOGY, PHYSIOLOGY, SEROLOGY, LONGEVITY, AND PATHOGENICITY OF CORYNEBACTERIUM SEPEDONICUM

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INTRODUCTION

During the study of ring rot of the potato in the United States and elsewhere during the last few years, a number of interesting and heretofore unreported properties of the causative organism *Corynebacterium sepedonicum* have been described. The main contributions to the knowledge of the nature of the disease and of the biology, morphology, cultivation, and systematic position of the ring-rot organism have been reported by Bonde (1, 2, 3), Dykstra (7), Eddins (9, 10), Racicot *et al.* (15), Savile and Racicot (16), Skaptason and Burkholder (17), and Stapp (20).

However, certain improvements still could have been made in the cultural methods and in the methods of determining the physiological properties of the organism. More information also has been needed regarding the serological properties, the viability and the virulence of this bacterium.

MEDIA AND CULTIVATION

In or on any of the media recommended for the cultivation and isolation of *Corynebacterium sepedonicum*, this organism grows relatively slowly. At the optimum temperature (22–25° C.) colonies clearly visible to the unaided eye appear in 8 to 12 days. This is a rather long time, and a shortening of the incubation period needed for the isolation or growth of *C. sepedonicum* seemed very desirable. After many old and new media were tried one was found that was particularly satisfactory. The composition of this medium ("4–d") was as follows:

Bacto peptone	3 g.
" tryptose	3 "
" yeast extract	3 "
Dextrose	3 "
Distilled water	1 liter
pH 7.0	

It was found later that maltose, and to a certain degree lactose, also supported the growth of *C. sepedonicum*, without any appreciable lowering of the pH of the medium during incubation which may accelerate the death of the bacteria. Therefore dextrose has frequently been replaced with maltose (medium "4–m"). In the routine work "bacto technical maltose," as less expensive, was used in place of maltose C.P. For prolonged cultivation, as

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in stock cultures, one third of the maltose has been replaced by lactose (medium "4–m–l"), inasmuch as lactose also was found to be utilized slowly by *C. sepedonicum* with very slow and moderate lowering of the pH of the medium, even after several months of cultivation. Growth on medium "4" (without any available source of carbon also is relatively fast, but scant in volume.

Experience has shown that stock cultures, maintained at room temperature, survive much longer in a liquid medium than on a solid one. Agar slant cultures, 6 weeks old, contained only a few viable cells, while 6-month cultures, maintained on the 4–m–l medium, usually gave luxuriant growth on fresh medium. Milk and litmus milk also were found to be suitable media for stock cultures.

Potato- or carrot-extract media gave various results for Spieckermann and Kotthoff (19), Stapp (20), and Savile and Racicot (16). We have found that potato and carrot extracts are quite satisfactory as media, even without additions, provided the pH is adjusted and maintained at about 7.0. Some extracts are too acid to obtain any growth without an adjustment of the pH (16).

The isolation of *Corynebacterium sepedonicum* from infected tubers or stems of potatoes is quite easy on any of the recommended media. On some media, however, like the 4–d or 4–m, the growth is so accelerated that the time necessary for colonies to develop large enough for satisfactory isolation is reduced from about 9 days to only 5. It has been reported that on the medium recommended by the laboratories of the U. S. Department of Agriculture (7) growth is also fast and abundant. The preparation of this medium seems, however, to be somewhat more complicated than that of the one here described. In plate I, figs. 1–5, are presented photographs of 10-day-old cultures on some of the tested media.

If the tubers are already invaded by soft-rot bacteria, isolation is often virtually impossible because *Corynebacterium sepedonicum* is overgrown by rapidly and abundantly growing colonies of *Erwinia carotovora* or similar soft-rot organisms. It has been found in preliminary experiments that the addition of separately steam-sterilized sodium dichromate to the sterile medium 4–m–l in concentration 1:20,000 completely inhibited the growth of *E. carotovora*, while *C. sepedonicum* grew only slightly less abundantly than on the medium without sodium dichromate. The addition of sodium dichromate made it less difficult to isolate *C. sepedonicum* from tubers in an advanced stage of decomposition due to secondary invasion by the soft-rot organisms. On agar plates with medium 4–m–l alone, colonies of the bacteria causing the secondary infection were found to be present in varying numbers, but no colonies of *C. sepedonicum* appeared (Pl. I, fig. 7). On the same medium with the addition of sodium dichromate 1:20,000 colonies of *C. sepedonicum* appeared in considerable numbers in most of the tested cases (Pl. I, fig. 6). The size of the colonies and the speed of their growth

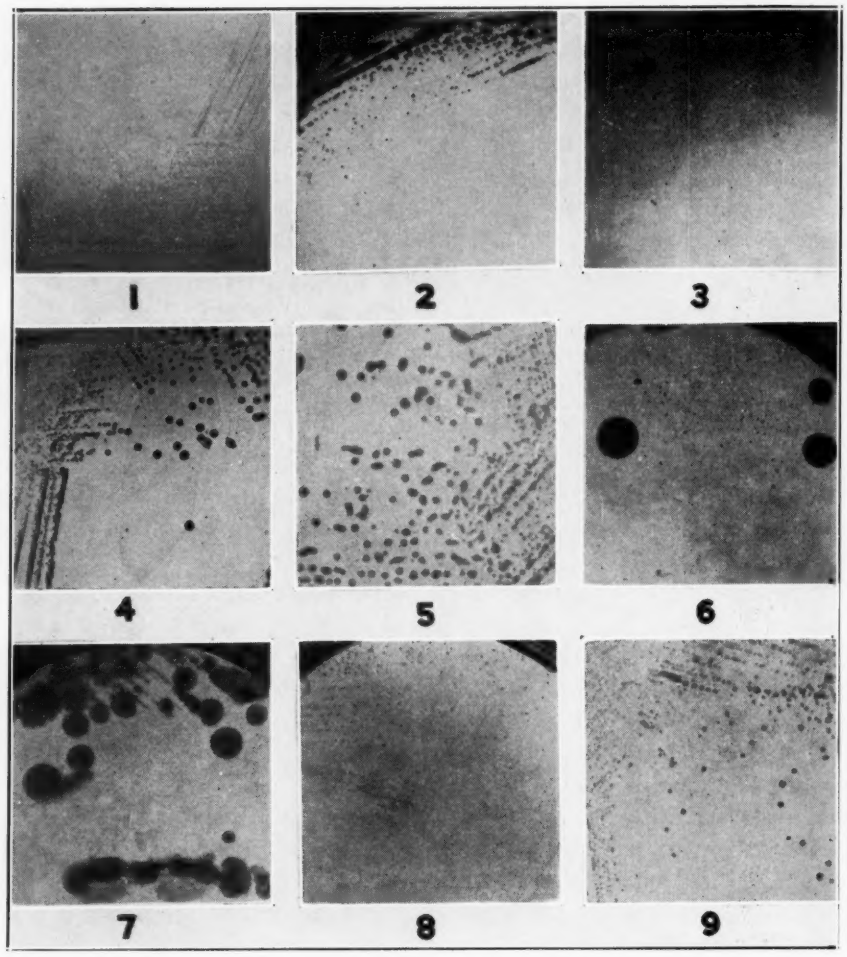

PLATE I

Corynebacterium sepedonicum cultures incubated 10 days at 22–23° C. FIG. 1. Nutrient agar plate. FIG. 2. Potato-extract agar plate with the addition of peptone, asparagine, and dextrose, pH 6.8–7.0. FIG. 3. Potato-extract agar plate, pH 6.8–7.0. FIG. 4. Medium No. 4-d agar plate, colonies of the "rough" type. FIG. 5. As figure 4, colonies of the "smooth" type. FIG. 6. Medium 4-m-l agar plate inoculated with aseptically removed and macerated tissue of a potato tuber with the symptoms of late ring rot with the secondary symptoms of advanced soft rot. Medium contains sodium dichromate in concentration 1: 20,000. Colonies of *C. sepedonicum* fairly numerous with few large colonies of soft-rot organism. FIG. 7. The same as figure 6 but medium without sodium dichromate. Inoculated with the same material as in figure 6. Colonies of *C. sepedonicum* absent. Colonies of soft-rot organism numerous. FIG. 8. Medium 4-m-l agar plate with the addition of sodium dichromate in concentration 1: 20,000. Inoculated with aseptically removed and macerated tissue of a potato tuber with early symptoms of ring rot without any secondary infection. Colonies of *C. sepedonicum* numerous but small. FIG. 9. The same as in figure 8 but medium without sodium dichromate. Colonies of *C. sepedonicum* well developed.

were less on the medium with dichromate than on the control medium without it (Pl. I, figs. 8, 9). This fact would indicate that at least a large proportion of the secondary invaders overcrowd not only because of greater numbers and faster growth but also because they exert a toxic effect on the *C. sepedonicum*. This toxic effect, however, apparently could be checked in the medium by the addition of small amounts of sodium dichromate.

Snyder and Lichstein (18) found that sodium azide is a bacteriostatic agent for Gram-negative bacteria, and Mallmann, Botwright and Churchill (14) demonstrated that both of the slowly oxidizing substances, namely, sodium azide and potassium dichromate, will inhibit the growth of Gram-negative bacteria and permit Gram-positive ones to grow. Wight (23) found that among various recommended slowly oxidizing agents, sodium dichromate had the widest range of growth inhibition for many Gram-negative bacteria.

THE UTILIZATION AND FERMENTATION OF VARIOUS SOURCES OF CARBON

While cultivating various strains of *Corynebacterium sepedonicum* on media containing added sugars, it has been observed frequently that these media were distinctly acidified. Spieckermann and Kotthoff (19), who first studied the utilization of various sources of carbon by *C. sepedonicum*, found that very little, if any, acid was produced from the various sources of carbon, and that litmus added to the media remained unchanged in color. Stapp (20) made no mention of any acid production from various carbon sources by this organism. Because a difference was found between the reports of these authors and our own observations, a more detailed study was undertaken regarding the utilization and fermentation of various sources of carbon.

Medium 4 was used as a base, having no carbon sources added, as previously stated. A solid medium was prepared by the addition of 2 per cent of agar. Brom-thymol-blue, as the pH indicator, was added to the medium in the concentration of 0.002 per cent. The reaction of the medium was adjusted to approximately pH 7.0. Carbon sources were added in the concentration of 0.5 per cent. Those that would decompose in the autoclave were sterilized by filtration and added aseptically to the medium. Comparative observations were made on the cultures growing on solid media and in liquid media. Ten strains of *C. sepedonicum* were used, some several years old and the others isolated a few months or weeks before. Because the organism grew slowly, the fermentative changes in the medium frequently appeared very late.

Among all the tested sources of carbon (Table 1) it was mainly the monosaccharides that were fermented readily with distinct acidification of the medium. A medium containing dextrose was acidified faster when in the form of agar slants than as a liquid. The pH of the agar slants reverted within 20 days from 6.0 to 6.5 to 7.0, depending on strain. The pH of the liquid medium with dextrose remained near the 6.0 level until the close of

the experiment, or 50 days. A medium containing sucrose underwent nearly the same pH changes as the medium with dextrose. Maltose and lactose, also, were fermented, but more slowly; liquid media containing these sugars became acid within 50 to 80 days from inoculation, and in 100 to 130 days the acidity of the media gradually diminished. Of the alcohols tested, mannitol alone was fermented; the result was the production of acid in liquid medium within 40 to 50 days. Salicin was fermented only by the growth of the secondary colonies, which regularly appeared within 30 to 40 days of incubation. The resulting change of pH occurred suddenly due to the presence of an adaptive enzyme together with the sudden development of quite luxuriant growth of the secondary colonies on agar slants. In the liquid medium containing salicin the pH changes were less pronounced. It appeared that the secondary colonies were variants that were able to utilize salicin. The primary growth was scant, the same as on the check medium without any carbon source added. The secondary growth was abount as abundant as on the media containing any of the readily utilized sources of carbon. The two consecutive transfers from the secondary colonies to fresh medium with salicin failed to result in the accelerated fermentation of salicin pointing to the fact that the fermentative adaptation was only temporary.

GROWTH AND VIABILITY IN LITMUS MILK

Particularly interesting was the type of growth in litmus milk. Fat-free milk with the addition of *lacmoid indica* was sterilized in live steam for 3 consecutive days, tested for sterility, inoculated with various strains of *Corynebacterium sepedonicum* and incubated at approximately 25° C. During the first 40 days of the incubation the only sign of growth was the reduction of the litmus for about 1 cm. from the bottom of the test tube. Between 40 and 60 days after inoculation, all strains, except one that was also serologically atypical, reduced litmus completely throughout the medium, and developed a transparent yellow serum zone 1 to 2 cm. deep on the top. Up to the end of 130 days of cultivation no other changes occurred, except for the slow widening of the serum zone. This zone was viscid and gelatinous, reminding one of the vaseline seal used on anaerobic media. It apparently reduces the evaporation of the milk, which evaporated less than half as fast as other liquid media. Jensen (13) found that such a zone is produced in milk by various saprophytic soil corynebacteria and mycobacteria, and is one of the very important differential characteristics of those microorganisms. The milk underneath at first appears coagulated; but, when the gelatinous serum zone is removed from the top, it is found to be normal and not coagulated. It has been found that litmus milk can be used successfully for the maintenance of stock cultures of *C. sepedonicum*. Litmus remained reduced for at least 8 months in the milk cultures of this organism left undisturbed at room temperature. In some of the cultures in which the gelatinous serum zone was broken up by shaking,

the litmus became oxidized. Transfers made sometime later from the milk cultures in which litmus was reduced gave very abundant growth, while transfers from cultures in which litmus became oxidized gave no growth, or reproduced only very few cells. It also has been found that when the number of viable cells of *C. sepedonicum* in litmus milk culture begins to decrease, the oxidation of the litmus in the top layer of the medium takes place. This is the best indication that the stock cultures should be transferred to a fresh medium. Stock cultures maintained in the liquid medium "4–l" (with lactose) survived nearly as well as those in litmus milk, while in liquid medium 4–m (with maltose) less than half of the strains survived during the same period of time and under the same conditions.

SURVIVAL IN THE SOIL

There is a question whether or not *Corynebacterium sepedonicum* can survive the winter in the soil. Most American authors, Bonde (2, 3, 4), Eddins (9 and 10), Eide and Rose (11), Vaughn and Leach (22), Dykstra (7) and Dykstra *et al.* (8), claim that *C. sepedonicum* cannot overwinter in the soil. This conclusion has been based on the fact that potato seed pieces free from ring-rot infection, planted in soil in which infected plants formerly were grown, gave a healthy crop. Stapp (20) found that *C. sepedonicum* left buried in the field in soil, after inoculation on sterile media and introduction into sterile soil, survived from November to May. In our experiments pure cultures of *C. sepedonicum* were added to sterile samples of Caribou and Washburn loams. These samples were placed in 24 one-quart glass jars with glass tops and rubber gaskets; 400 g. of air-dry soil was put in each jar. To each alternate sample 10 g. of powdered calcium carbonate was added. The preparations were then sterilized for several hours at 20 pounds pressure and inoculated each with 25 ml. of a water suspension of *C. sepedonicum*. The amount of sterilized water then added to various jars varied from 25 to 100 ml. per jar. The jar clamps were closed and the jars placed in clay drainage tiles that were buried in the field at Aroostook Farm at a depth of eight inches beneath the surface of the soil on October 28, 1941. Upon removal in May, 1942, the soil in the jars was tested bacteriologically. Of the 24 jars buried in the soil, 15 were found to contain soil contaminated with various Gram-negative bacteria, which, being also non-spore-forming, obviously had penetrated the jars past the rubber gaskets (the jars not being vacuum sealed). The soil in 4 jars with Caribou loam was sterile, even *C. sepedonicum* was absent. The soil in 5 jars contained *C. sepedonicum* alone. Those 5 jars yielded cultures that were all identified as *C. sepedonicum* on the basis of their morphological, biological, serological, and pathogenic properties. The reisolated strains were tested in regard to their virulence and were found to be pathogenic causing typical ring-rot symptoms when used for inoculating potatoes. To 4 of these 5 soil samples calcium carbonate had been added. No definite influence or soil type or of water content of the soil was noticed.

TABLE 1.—*Utilization and fermentation of various sources of carbon*

Carbon source	Utilization as estimated by the amount of growth[a]			Utilization as estimated by the change in pH on medium 4 (Snieszko and Bonde—1941–42)					
				In liquid medium[b]					On agar slants
	Spiecker-mann and Kotthoff 1914	Stapp 1930	Snieszko, Bonde 1941–42 (within 10 days)	10 days	20 days	30 days	40 days	50 days	(Medium 4 with sources of carbon)
l-Arabinose	++++	+	++++	+++ ++++	++++	++++	++++	++++	pH 6.2–6.4 in ten days
Xylose	++++	+	++++	++++	++++	++++	++++	++++	pH below 6.0 in ten days
Rhamnose	++++	+	0	0	Slightly alkalized				As check
Dextrose[c]	++++	++++	++++	++	+++ ++++	++++	++++	+++ ++++	pH 6.0 within ten days; within next ten days the pH of most strains slowly returned to 6.5–7.0
Galactose	++++	++++	++++	++ +++	++++	++++	++++	++++	pH 6.4–6.6 in ten days; after 20 days returned to original pH
d-Levulose	++++	++++	++++	++++	++++	++++	++++	++++	pH 6.0 in ten days
Saccharose[c]		++	++++	0	+++	+++	++++	++++	pH 6.0 in ten days; in 40 days returned to 6.8
Lactose[c]		++	+	0	+	+	++	++[e]	As check
Cellobiose			+++	+	++	+++	++++	++++	pH 6.4–6.6 in 20 days
Maltose[c]		++	++++	+	++	++	++ +++	+++[e] ++++	pH 6.5 in 20 to 30 days
Raffinose		+	0	0	0	0	Slightly alkalized		As check
Inulin		+	0	0	0	0	Slightly alkalized		As check
Starch, soluble	+	0	±	0	0	0	Slightly alkalized		As check
Dextrin		+	0	0	0	0	Slightly alkalized		As check
Glycogen		0							
Ethyl alcohol			0	0	0	Slightly alkalized			As check
Glycerol	++	+	+	Some strains produced some acid; some strains were slightly alkalized					Some strains slightly alkalized, some slightly acidified
Adonitol			0	0	0	0	Slightly alkalized		As check
d-Mannitol[c]		++	+++	+	++	++	+++	++++	Trace of acid in 10 to 20 days; later returned to original pH
Dulcitol		++	0	0	0	0	0	0	As check
Sorbitol			0	0	0	0	0	0	As check
Salicin[c]			+(+++[c])	0	0	0	++	++	pH 6.0 or below in 30 to 40 days
Esculin[d]			+	++	++	++	++	++[d]	Acidified in 10 days
Inosite			0	0	0	Slightly alkalized			As check
Check (medium 4 alone)			Growth scant	0	Slightly alkalized				Slightly alkalized in 20 days

[a] In these 3 columns, 0 = no growth, ++ = moderate growth, +++ = good growth, ++++ = abundant growth.
[b] In these 5 columns, 0 = no change in pH, or growth the same as in check, + = pH 6.8 to 7.0, ++ = pH 6.5 to 6.8, +++ = pH 6.2 to 6.5, ++++ = pH 6.0 or below.
[c] More details in text.
[d] pH not determined.

Data presented in table 2 show that those cultures of *Corynebacterium sepedonicum* reisolated from soil and agglutinated by the specific agglutinating serum gave high incidence of infection. Those that did not agglutinate were very slightly or not at all virulent.

This experiment is in agreement with the results obtained by Stapp (20); the fact, however, that *Corynebacterium sepedonicum* was isolated

TABLE 2.—*Pathogenicity of Corynebacterium sepedonicum reisolated from soil overwintered in the field*[a]

Cultures reisolated from soil samples		Infection occurring
No.		*Per cent*
10	Morphological, biological and serological characteristics typical for *C. sepedonicum*	90
11		40
18		100
20		50
21		100
6	Morphological and biological characteristics typical, but serologically atypical	0
14		10
22		10
	Macerated tissue of the potato tuber with symptoms of ring rot	100

[a] Pure cultures of *Corynebacterium sepedonicum* were added to sterilized soil in Mason jars. The jars containing the cultures were placed in clay tiles submerged in the field and then covered with eight inches of field soil. The cultures remained in the field from October 28 until May 25, and were then removed and taken to Orono where the organism was reisolated and grown on artificial media.

from none of the contaminated jars would suggest either that *C. sepedonicum* cannot survive long in the soil in the presence of other bacteria, or that the isolation of *C. sepedonicum* from a mixed bacterial flora was not possible due to the overcrowding of *C. sepedonicum* on the agar plates during the attempted isolation. This finding would be in agreement with the results obtained in Minnesota and presented by Dykstra (7), who reported that *C. sepedonicum* in nonsterile soil survived for a shorter time than in sterile soil. Data presented in table 3 also indicate that the ring-rot organism dies off faster in badly disintegrated tubers in which secondary invaders are present, than in tubers containing *C. sepedonicum* in a pure state.

Another indication that *Corynebacterium sepedonicum* cannot survive long in the presence of certain other microorganisms was obtained when a number of badly disintegrating potatoes were tested microscopically and culturally for the presence of the ring-rot organism. In all of the tubers tested *C. sepedonicum* was found to be present in preparations stained with the Gram method. Secondary invaders also were seen in the preparations in varying numbers, but the amount of *C. sepedonicum* was always vastly predominant. On the medium 4–m–l agar plates, with the addition of sodium dichromate 1 : 20,000 and without it, *C. sepedonicum* grew only in few cases, these being on the medium with the dichromate. The number of colonies of the contaminants on the medium with the dichromate was always moderate

[c] More details in test.
[d] pH not determined.

and not sufficient to suppress entirely the growth of *C. sepedonicum*. This experiment, together with the infection experiments and the results reported by Dykstra (7), would indicate that *C. sepedonicum* dies quickly in the presence of other microorganisms.

TABLE 3.—*The effect of aging of ring-rot inoculum on its pathogenicity*[a]

Inoculation number	Material used as inoculum	Seed pieces inoculated	Infected plants resulting from inoculation
		Number	*Per cent*
1	Control. Macerated ring-rot tubers used immediately for inoculations	10	100
2	As No. 1 but stored 13 days	10	0
3	Badly disintegrated ring-rot tuber macerated in sterile water, used immediately for inoculation	20	0
4	Control. Macerated not disintegrated ring-rot tubers used immediately for inoculations	20	70

[a] Studies conducted in the greenhouse.

EXPERIMENTS ON VIRULENCE

On January 29, and July 1, 1942, cut potato seed pieces of the Green Mountain variety, which was found by Bonde *et al.* (5) to be very susceptible, were inoculated and planted. The inoculum was 10 strains of *Corynebacterium sepedonicum,* one half isolated in September of 1942 and the other half during 1940. Cultures grown 22 days on the solid medium 4–m were washed off with water to form suspensions in which the cut potato seed pieces were dipped shortly before planting. Potato plants were grown in a greenhouse at Presque Isle at the average temperature of 65° C. Observations were made on April 23, May 17, and September 11, 1942, and were based on the presence or absence of external symptoms of the disease. The results indicate (Table 4) that the cultures of *C. sepedonicum,* maintained for longer periods on artificial media, were losing their virulence markedly.

Between the results described in the two tests of table 4 the lapse of time was 6 months. It shows that all the cultures were losing their virulence markedly, even in such a short period. This fact may explain why certain workers have not secured successful results from inoculation with the cultures grown for a longer period on artificial media.

SEROLOGICAL EXPERIMENTS

Two rabbits were immunized by repeated weekly intravenous injections of suspensions of *Corynebacterium sepedonicum.* No pathogenic effects were noticed in the rabbits, even after injecting into them large doses of the living bacteria. The agglutinating sera obtained, as in the case of nearly all Gram-positive bacteria, had low titre, namely:

Serum 1. 1:40 complete agglutination
1:80 partial agglutination

Serum 2. 1:5 complete agglutination
1:10 partial agglutination
1:20 partial agglutination

TABLE 4.—*Pathogenicity of strains of Corynebacterium sepedonicum*[a]

Strain number[b]	Infection occurring[c]	
	First test[d]	Second test[e]
	Per cent	*Per cent*
2	90	10
4	0	20
8	0	0
8a	10	0
10	80	.....
11	90	20
12	90	0
13	100	40
14	100	70
15	100	.....
Control; pulp from infected potatoes smeared on seed pieces	70	100
Control; water contaminated with bacteria from infected tubers	87	100

[a] These strains had been studied also regarding their morphological and physiological properties.

[b] Strains 2, 4, 8, 8a, and 10 are older strains isolated by Bonde in 1940. Strains 11 to 15 were isolated by Snieszko in September, 1941.

[c] Ten freshly cut seed pieces of the Green Mountain variety were inoculated for each test by being dipped in water suspension of the bacteria and then planted immediately in the greenhouse. The bacteria were grown on agar in "French square" bottles, and the bacteria from each bottle were suspended in 200 cc. of tap water.

[d] The seed pieces were inoculated January 29, and the symptoms on the plants recorded May 17, 1942.

[e] The seed pieces were inoculated July 1, and the symptoms noted September 11, 1942.

All 19 strains of *Corynebacterium sepedonicum* isolated in the course of the last few years were tested. The test was made with the saline suspensions of the bacteria with an addition of 0.3 per cent of phenol. All tests were incubated for 48 hours at 25° C. The results were recorded after 12, 24, and 48 hours. The tests were actually completed within 12 to 24 hours, so that further incubation was proved to be superfluous. All suspensions were checked with the pure saline, normal rabbit and sheep sera, and the rabbit-agglutinating sera against entirely different bacteria belonging to the genus Salmonella.

None of the tested strains of *Corynebacterium sepedonicum* agglutinated with the physiological saline solution alone, two strains (7 and 8a) agglutinated with normal and heterologic sera, and one (No. 16) was not agglutinated by the specific sera. One strain (No. 4) was agglutinated by serum No. 1 in dilution 1:10 but not by serum No. 2. Strains 8a and 16 were somewhat atypical in their other properties also. The rest of the strains

were agglutinated approximately up to the titre of both sera. This would indicate that *C. sepedonicum* forms a quite uniform serological group, and that the agglutination test may be used for the identification of this bacterium.

Tipograf (21) described a serological method of diagnosis of the ring-rot organism. The description of the method found in an English abstract "does not seem reasonable," according to Burkholder (6). The vascular tissue of the potato tuber in the advanced stage of infection is filled with *Corynebacterium sepedonicum* and, therefore, it could be used as an antigen in the agglutination test. The tests made with the available sera did not, however, give any promising results, because in a macroscopic agglutination test floccules of tissue could not be distinguished from the agglutinated bacteria. In the microscopic agglutination test the differences between the tests and controls were so insignificant, if indeed there were any, that the test appeared to have no value.

Israilski and Artemieva (12) recommended as reliable the precipitation test for the diagnosis of tomato plants infected by *Corynebacterium michiganense*. However, the preliminary precipitation tests, which we made with the extracts of *C. sepedonicum*, gave negative results.

TERMINOLOGY AND SYSTEMATIC POSITION OF CORYNEBACTERIUM SEPEDONICUM (SPIECKERMANN AND KOTTHOFF) JENSEN

Jensen (13) suggested that the bacterium causing the ring rot of potatoes should be clasified as a *Corynebacterium*. Skaptason and Burkholder (17) found that it "has all the characteristics of a *Corynebacterium.*" A study of the morphological and physiological characteristics of the bacteria, isolated from potatoes affected with ring rot, also has convinced the writers that the most proper systematic position of this organism is in the genus *Corynebacterium*. It has been found that the growth in milk is very typical with the production of the gelatinous serum zone that is considered by Jensen (13) to be an important characteristic. The only difference, a very insignificant one, between the properties of the strains of *C. sepedonicum* in our description and the description given by Stapp (20) is that none of our strains liquefied gelatine, while some of those freshly isolated by Stapp liquefied it slightly. Spieckermann and Kotthoff (19), on the other hand, did not find any liquefaction of gelatine.

The evidence at hand and the descriptions given by other authors support the view that the organism causing the ring rot of potatoes should be classified as *Corynebacterium sepedonicum* (Spieckermann and Kotthoff) Jensen.

SUMMARY

A new medium is recommended for the isolation and cultivation of *Corynebacterium sepedonicum*. As nitrogen sources this medium contains Bacto peptone, Bacto tryptose and Bacto yeast extract, and as carbon sources, dextrose, maltose, and occasionally lactose.

For the maintenance of stock cultures, liquid media are more suitable than agar media. Litmus milk is a splendid medium for identification and for the cultivation of stock cultures.

The best sources of carbon are monosaccharides such as arabinose, xylose, dextrose, galactose and levulose; rhamnose is not utilized. Disaccharides are utilized more slowly. Among other sources of carbon, mannitol is the best. All sources of carbon which are utilized are decomposed with a slow and sometimes only temporary increase of the acidity of the medium.

The new medium with the addition of sodium dichromate in concentration 1:20,000 can be used for the successful isolation of *C. sepedonicum* from potato tubers having symptoms of soft rot due to secondary infection with *Erwinia carotovora.*

Pure cultures of *C. sepedonicum* when added to sterile soil and left buried in the ground during the winter survived and retained their virulence.

Cultures of *C. sepedonicum,* if maintained on laboratory media, gradually lose their virulence towards potatoes. Infection experiments in which macerated ring-rot tubers were stored for 13 days before inoculation gave negative results. Also, badly decayed tubers of ring-rot potatoes were not infectious.

C. sepedonicum is weakly antigenic for rabbits, producing agglutinating sera with low titre. All tested strains of *C. sepedonicum* were cross-agglutinated approximately up to the titre of all sera.

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ACUTE AND CHRONIC SYMPTOMS IN TOBACCO MOSAICS

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INTRODUCTION

In the tobacco mosaics the disease reactions are not uniform in all leaves of a given plant. In 1929, McKinney (4) reported and illustrated the striking chlorotic reaction, referred to as the "oak-leaf" pattern by later workers. This appears in tobacco leaves advanced beyond a certain stage of development at the time of inoculation with a yellow-mosaic mutant from the virus of tobacco common mosaic. In 1932, Holmes (3) reported 5 symptom reactions in tobacco plants infected with the tobacco common-mosaic virus.

Studies on the ring-spot disease of tobacco led Valleau (13) and also McKinney (5) to take the view that the disease manifests an acute phase and a chronic phase, and that the so-called acquired immunity in this disease may be regarded as the chronic phase. McKinney (5) took the position that in the tobacco ring-spot disease, the low level of symptom expression in the chronic phase reflects natural resistance in the host. Pursuing this working hypothesis further, it became evident that the ring-spot virus is essentially a mosaic virus when it is studied in a more susceptible host (8). Accordingly, it was concluded that symptom reactions with tobacco mosaics should be reassessed, as many observations over a period of years indicated that acute and chronic disease reactions obtain in certain of these diseases also. Results of recent experiments on this point have been summarized by the writers (8).

This paper deals chiefly with the succession of symptoms occurring in the natural course of a yellow mosaic in tobacco; it gives also certain results of similar studies on common mosaic.

MATERIALS AND METHODS

The host plants used were Samsun (Turkish) tobacco and one F_1 backcross generation (*Nicotiana tabacum* × *N. longiflora* × *N. tabacum*). The viruses used were wild-type common-mosaic virus (Nicotiana virus 1) (6), yellow-mosaic mutant virus *BSY* (6), and a yellow-mosaic virus (white-mosaic virus) (7) obtained from W. D. Valleau.

Except when otherwise stated, the inoculations were made with fresh native virus extract on a small amount of cotton inserted into the axil of a leaf near the base of the stem by means of a needle.

Tests have been carried out during all parts of the year, but the results summarized in figures 1, 2, and 3 are from tests conducted in a greenhouse during late summer and early autumn. Temperatures were held as near 22.5° C. as possible, but day temperatures usually were higher, as bright

[1] Acknowledgment is due Matthew Koerner for assistance in conducting the tests.

days prevailed during the test. Plants were grown in earthen pots of ample size. A composted soil was used throughout.

Throughout the paper, the term *acute* reaction denotes severe chlorosis and/or early death of the diseased tissue. The term *chronic* reaction denotes invaded tissue that may or may not express obvious symptoms, death

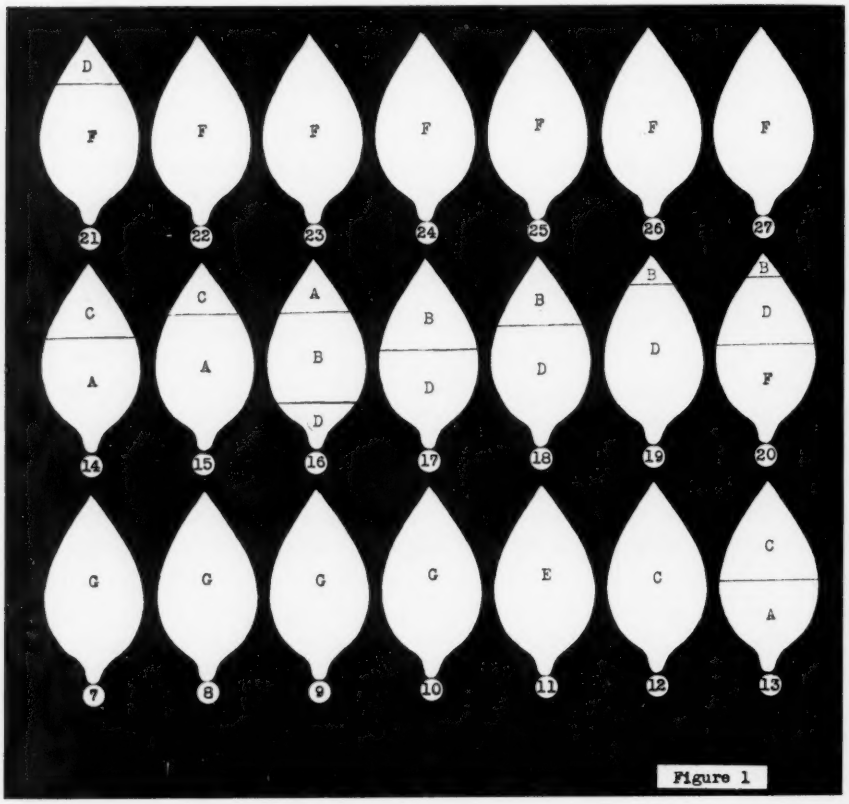

FIG. 1. Diagrams of mature leaves of virus-infected Samsun (Turkish) tobacco numbered from base of plant, to show the progressive appearance of the several symptom types in each leaf. Where two or more symptom types occur on the same leaf, their approximate boundaries are indicated by a line. The 7 symptom types are lettered A to G in the approximate order of their appearance. All leaves were made of uniform size in the diagram for convenience, but actually they were of different sizes as shown by the curve for mature leaves in figure 4. The symptom types are described on pages 1049 and 1050. Leaf numbers correspond with those in figures 2 and 3.

of tissues following chlorosis only after a long delay or under environmental conditions that are more extreme than those obtaining for the expression of acute reactions in the same plant.

RESULTS

Yellow Mosaic

In Samsun tobacco plants of moderate size the symptoms of yellow mosaic are summarized graphically in figure 1. Type symptoms are illustrated in

figures 2 and 3. In figure 4 the length of each leaf in mm. at the time of vein-clearing and at the time each leaf finished its growth, and the lengths of the mature leaves of a disease-free plant are graphed for comparison.

The several degrees of disease reaction or symptom types that appeared in the leaves assumed 2 gradient series. Proceeding from the most acute

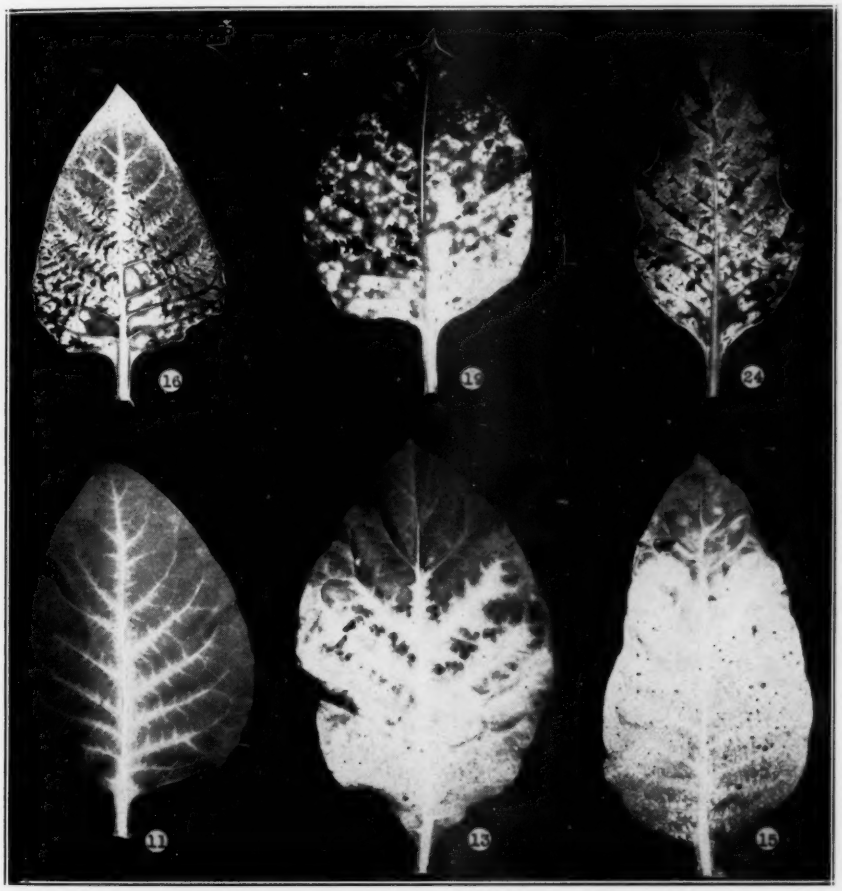

FIG. 2. Leaves of Samsun tobacco showing the types of symptoms referred to in the diagrams in figure 1. These leaves represent samples that correspond closely with the leaves diagrammed in figure 1, and the leaf numbers correspond with those in figure 1. Photographs were taken before necrosis appeared.

reaction (symptom type A), one gradient proceeded up, the other down the stem; progress being greater in the upward direction, especially during winter months. For sake of convenience, the entire series is divided into 7 rather characteristic zones of disease reactions or symptom types, and lettered A to G. Five types, designated A to E, inclusive, comprise the acute phase as follows:

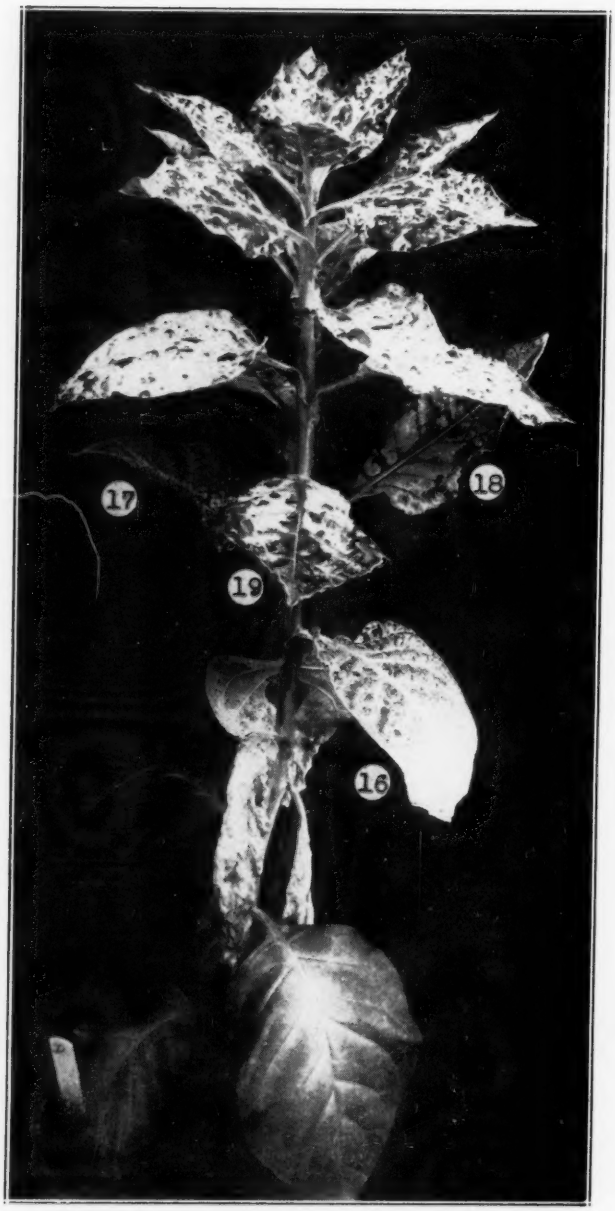

FIG. 3. Samsun tobacco plant with yellow mosaic. Leaf numbers correspond with those in figure 1. Necrosis and death occurred in leaves 12, 13, and 14, in tips of leaves 15 and 16, and in most of zone D in leaves 17, 18 and 19. Plant was inoculated in the stem near the base. $\times \frac{1}{4}$.

Symptom type A appeared first as vein clearing (faint chlorosis bordering the small vascular bundles). This was the first sign of disease in the plant, and it appeared approximately 8 days after inoculation, reaching its limits in about 8 hours, after which there was a rapid development of acute

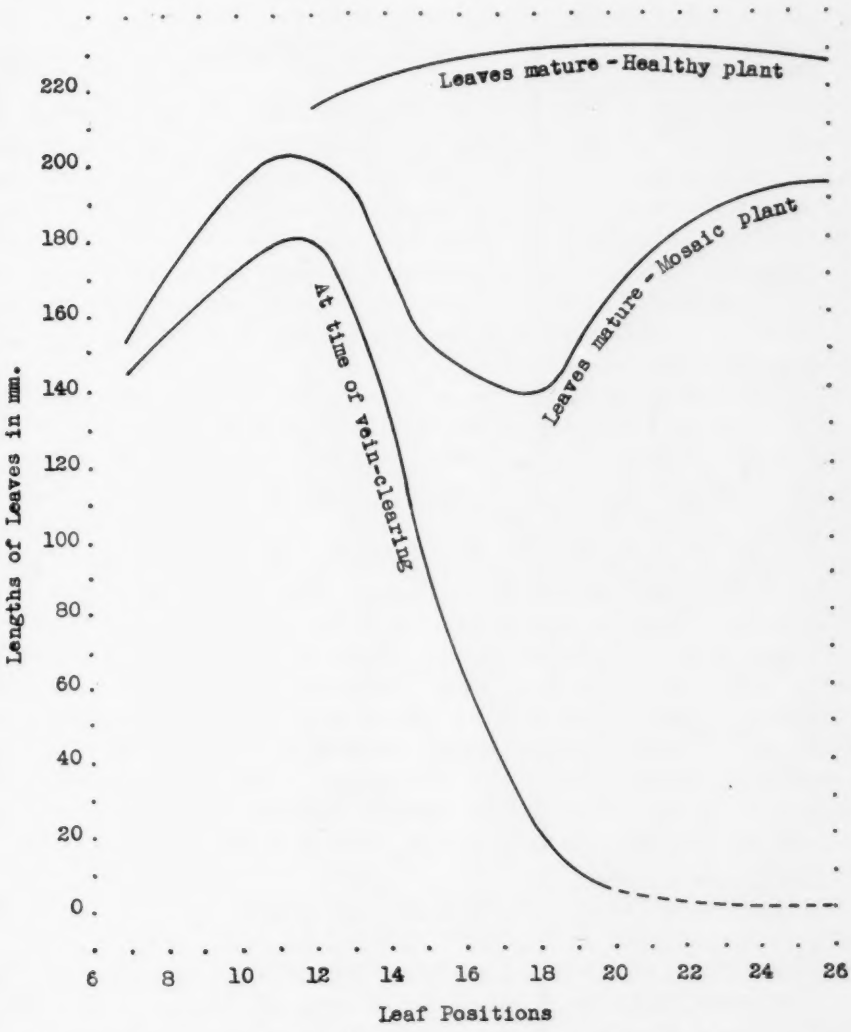

Fig. 4. Smoothed curves showing the length of leaves on a healthy Samsun tobacco plant and on a plant with yellow mosaic. The leaf position numbers correspond with those in figure 1.

chlorosis (severe yellowing) throughout most of the parenchyma within the vein-cleared zone. The original vein-cleared margins along the small veinlets tended to remain light-green, producing a netted effect illustrated in leaf 15 (Fig. 2). Acute necrosis was the final symptom. This represents the most severe reaction in the acute phase.

Symptom type B manifested no vein-clearing, but the development of severe chlorosis and acute necrosis resembled that in type A, except that wide light-green vein bands occurred along the large veinlets, as illustrated in leaf 16 (Fig. 2).

Symptom type C is characterized by chlorosis (severe yellowing), which progresses from the midrib, primary, secondary, and tertiary lateral veins, causing the "oak-leaf" pattern. Acute necrosis was characteristic (leaf 13, Fig. 2).

Symptom type D developed in very young tissue at the time chlorosis appeared in types A and B, and as type D developed, it tended to remain green for a considerable period, but later chlorotic and yellow patches appeared and caused the mottled effect illustrated in leaf 19 (Fig. 2). This reaction was followed by acute necrosis, especially in the yellow areas. This symptom type blends into the chronic-mosaic symptom type F.

Symptom type E is similar to type C, severe chlorosis occupied parenchyma tissue along the midrib, primary, secondary and tertiary lateral veins, acute necrosis was irregular (leaf 11, Fig. 2).

The chronic phase (typical mosaic mottling), represented by symptom type F (leaf 24, Fig. 2) continued in all subsequent leaves and there was no necrosis under minimal conditions that favored necrosis in the older leaves (types A to E). Typical mosaic commenced in leaf tissue that was differentiated near the time of vein-clearing. In growing leaves with the chronic yellow mosaic, the green mottled areas frequently developed "secondary" light-green and yellow areas as described in another paper (7), also the yellow areas usually took on a greenish color due to new cells that contained chlorophyll, or to the development of chlorophyll in cells that were devoid of it earlier. Leaves showing typical mosaic grew slowly and never reached the size of corresponding normal ones. During extremely hot, bright periods of mid-summer, necrosis sometimes was an attendant symptom in typical mosaic leaves that had attained about $\frac{1}{2}$ or more of their growth. Necrosis in these leaves, however, is dependent on more extreme temperature and light conditions than it is in older leaves manifesting symptom types A to E.

After the onset of typical mosaic, the young leaves tend to exhibit the most intense mottling in the older tissues (distal region), and sometimes, especially during dark weather, more than one-half of the proximal portion of these leaves will be free of apparent symptoms. The progress of mottling in these areas resembles that occurring in tissue showing symptom type D, except that necrosis has not been identified as an attendant symptom.

Symptom type G manifests mild chlorosis, which spreads almost solidly from the primary lateral veins, involving all parenchyma as it progresses. Acute necrosis was not an attendant symptom; however, senility and final death of tissue was hastened. This may be regarded as a chronic manifestation in old leaves nearing their decline.

In young plants with the 8th leaf 42 mm. long at time of inoculation,

and also in younger plants, the acute chlorotic-necrotic stage was so severe that many plants were killed, and those that survived were very weak. Death seemed to result chiefly from the complete necrosis in leaves that exceed 10 mm. in length, as acute necrosis did not occur in the more resistant small leaves or in the tip of the stem.

Each disease reaction or symptom type tended to be associated with leaf tissue that had reached a particular stage of development at the time of infection. Chlorosis and necrosis occurred in acute form in leaves that ranged in length from near 179 mm. to 3 mm. (Nos. 12 to 21, inclusive, Figs. 1 and 3) at the time of vein-clearing. From the curves in figure 4, it will be observed that leaf 12 had attained about 83 per cent of its final length, as computed on a basis of the corresponding leaf on a healthy control plant. In the writers' abstract (8) this figure was given as 66.66 per cent. The former figure seems to be more nearly correct, but further study will doubtless result in a change in this value as the final accuracy of such comparisons depends on many observations. Some of the leaves manifested 2 and occasionally 3 types of disease reaction, and each type tended to be associated with tissues that had reached approximately the same age in the several leaves.

Individual plants show variations, and symptom types may occupy more or fewer leaves than are shown in figure 1, depending on the age of the plant at the time of inoculation, the amount of light, and the temperature after the onset of symptoms. Observations on several tests have indicated that the acute reactions are accentuated and hastened by bright sunlight and high summer temperatures and delayed and ameliorated by dark weather. No essential differences were noted between the two yellow-mosaic viruses used.

In certain interspecific crosses, the sequence and expression of symptoms induced by yellow-mosaic viruses of the *BSY* type differ from the situation in Samsun tobacco. One F_1 backcross generation (*Nicotiana tabacum* × *N. longiflora* × *N. tabacum*) obtained in 1941 is of special interest. At 21 to 24° C. the plants developed primary necrotic lesions abundantly on wiped leaves, a semi-mild form of secondary necrosis involved the midribs and/or the lateral veins of the wiped leaves and a few of the chronic-mosaic leaves. The portion of the stem cortex in the zone of the necrotic midribs of lower leaves also developed mild necrosis. Typical acute and chronic symptom types of yellow mosaic, similar to those in Turkish, occurred in all the plants. The progress of the necrosis was not increased when the plants were subjected to 31° C., and chronic yellow mosaic continued at 21 to 24° C. until the plants were matured.

When the chronic-mosaic leaves of these plants were wiped with the same virus employed in the initial inoculation (virus *BSY*), no signs of local necrosis or any other manifestation resulted. Thus it is apparent that the primary necrotic reaction that is characteristic of healthy leaves cannot

be induced in the chronic diseased tissue by reinoculation with the same virus.

Common Mosaic

Distinction between acute and chronic symptoms is less striking in common mosaic than in yellow mosaic. The progress of the symptoms was slower than in the yellow mosaic, and this influenced the number of leaves in the acute phase, as well as the onset of the typical chronic symptoms.

Tobacco plants grown in the field frequently show necrosis in the older leaves when infection by common-mosaic virus occurs during midsummer. This acute reaction, referred to as blister by McMurtrey (10) and as burning by Valleau and Johnson (14), occurs rather commonly in infected plants, especially during hot weather.

Virus Assays

The present study did not include extensive assays to determine the progress of virus in the tissues of the several symptom types. Preliminary assays, however, were made on certain tissues from yellow-mosaic plants, and the results are summarized.

When vein-clearing was just faintly evident in tobacco leaves 130 to 150 mm. long, small leaves 5 mm. long and on the same plants, were removed, weighed, pulped in a mortar and diluted 50 times in M/10 phosphate-buffered solution at pH 7. This inoculum was wiped on carborundum-dusted leaves on 5 Samsun (Turkish) tobacco plants. Since all of the test plants developed yellow mosaic it is evident that virus had reached the very small leaves by the time the first symptoms appeared in larger leaves. After the onset of the chronic yellow-mosaic symptoms, assays were made on (a) leaves ranging in length from 1 to 5 mm., (b) the proximal half of leaves 55 to 60 mm. long and (c) the distal half of leaves used in sample (b). No signs of mosaic mottling were present in sample (b), but there was much mottling in the tissue used in sample (c). All tissues were collected from the same plants. The samples were prepared as in the previous test and diluted to 10^{-4}. Each sample was wiped on 20 primary leaves (10 plants) of *Phaseolus vulgaris* L., var. Scotia. Samples (a), (b), and (c) induced 2.7, 20.5, and 265.7 local lesions per plant, respectively. In a test reported previously (7) there was less virus activity in chronic-mosaic tobacco leaves 12 mm. long than in the larger leaves tested.

When vein-cleared leaf tissue had become almost completely chlorotic, but not necrotic, a comparison was made with leaf tissue at about the same stage of development, but with chronic-mosaic mottling. The leaves were from different plants. The tissues were processed as in the assays mentioned above, diluted to 2×10^{-5}, and the resulting inoculum was wiped on 20 primary leaves (10 plants) of Scotia beans. The vein-cleared chlorotic sample induced 21.6 lesions per bean plant, whereas the chronic-mosaic sample induced only 4.8 lesions per bean plant.

DISCUSSION AND CONCLUSIONS

The results of these studies indicate that the natural course of tobacco mosaic in a given plant of Samsun (Turkish) involves two major phases, an acute phase and a chronic phase. The acute phase comprises several intergrading types of expression, and all expressions tend to form an unbroken and natural sequence that seems to parallel more closely the natural growth phases occurring in the developing leaves, than it does virus movement with respect to vascular channels in relation to leaf phylotaxy.

On the basis of the several degrees of severity of the disease reactions in relation to the age of the leaf tissues, it seems apparent that natural resistance changes with the growth and development of the leaf tissues, and that the level of this resistance, at the time the tissues become infected, tends to determine the type of disease reaction.

Leaf tissues in the early stages of development and those that have reached maturity are regarded as more resistant than tissues in the intervening stages. The chronic-mosaic phase appears in leaves that are differentiated at about the time vein-clearing becomes evident and thereafter. These leaves become infected when they are very small. It is possible that the initial cells are infected when leaf differentiation takes place, as Sheffield (12) found virus in the primordial meristem dissected from the stem apices of tomato plants infected with tobacco common-mosaic virus and with Aucuba-mosaic virus, respectively. Resistance in the young tissues may be due to inability to maintain a high level of virus synthesis and/or to some condition that tends to retard the movement of virus.

It is well known that leaf tissues differing in age differ also in their physiology; it, therefore, is not surprising that they react differently to a virus. With the onset of disease, new physiological balances are set up (1, 2, 9, 11, 15, 16, 17) that, in some cases, may influence the symptom reactions of subsequent tissues. Furthermore, it seems highly improbable that a normal physiologic balance ever obtains in infected tissues, even though gross symptoms may be absent (2, 11). It is to be expected, therefore, that the several disease reactions occurring throughout the course of a disease will maintain a natural sequence that is essentially fixed for a given host, virus, and environment; and that necrosis will not occur in chronic diseased leaves when an additional dose of the virus already present is added.

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USTILAGO STRIAEFORMIS. II. TEMPERATURE AS A FACTOR INFLUENCING DEVELOPMENT OF SMUTTED PLANTS OF POA PRATENSIS L. AND GERMINATION OF FRESH CHLAMYDOSPORES[1]

K. W. KREITLOW[2]

(Accepted for publication April 1, 1943)

INTRODUCTION

Observations made in 1941 and 1942 showed that many plants of *Poa pratensis* L. in Pennsylvania pastures were infected with stripe smut *Ustilago striaeformis* (Westd.) Niessl. The extent of damage caused by this fungus is unknown; the disease, however, apparently is widespread, since practically every one of scores of pastures examined contained smutted plants.

The smutted plants, compared with healthy ones, were much reduced in size and seemed more adversely affected by the dry, hot mid-summer weather, when many of the diseased plants apparently die. Hence, it is to be expected that the heavy infestations of smut found in some pastures would result in considerable reduction in forage yields, particularly during mid-summer.

Since stripe smut is systemic and the causative organism may possibly be soil-borne, one of the most promising methods of control would seem to be in the selection or breeding of resistant varieties. The success of a breeding program is partly conditioned by the certainty with which infection can be produced in susceptible plants. A serious limitation to the approach of this problem is the frequent failure of fresh chlamydospores to germinate. The investigation here reported was undertaken to find, if possible, a treatment to hasten spore germination and determine the influence of temperature on development of smutted plants.

Examination of the literature revealed conflicting reports regarding germinability of the smut chlamydospores. Liro (5), inoculated different grasses with the organism but made no mention of encountering difficulties in securing germinable spores. Davis (1), stated that collections of *Ustilago striaeformis* obtained in May from *Poa pratensis* and *P. debilis* Torr. germinated without an after-ripening period; and Fischer (3) obtained 85 per cent germination of the chlamydospores from a collection of *U. striaeformis* on *P. pratensis* from Arlington, Virginia. On the other hand, collections of smut on *P. pratensis* tested by W. H. Davis (2), failed to germinate unless they were first subjected to an after-ripening period of 110–250 days. Germination of chlamydospores so treated, however, averaged only 35 to 60 per cent.

[1] Contribution No. 50, of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, State College, Pennsylvania, in cooperation with the Northeastern States.

[2] Associate Pathologist.

Pammel (6), reported that chlamydospores obtained from *Phleum pratense* L. germinated readily. Fischer (3), encountered no difficulty in germinating spores of a race of *Ustilago striaeformis* that occurs on *Agropyron trachycaulum* (Link) Malte, and *Elymus glaucus* Buckl. From chlamydospores of this race, he obtained cultures of *U. striaeformis* on artificial media. Kreitlow (4), recently reported a collection of smutted *Agrostis alba* L. that yielded fresh, germinable chlamydospores. Cultures secured from single chlamydospores growing on artificial media differed from those obtained by Fischer.

MATERIALS AND METHODS

Plants of *Poa pratensis*, infected with stripe smut, were collected from more than 75 pastures in Pennsylvania in 1941 and 1942. All diseased plants were separated into single tillers and transplanted to 4-inch pots of soil in a greenhouse. Chlamydospores from the smutted plants collected in each pasture were tested for germinability as soon as possible following collection.

Microscope slides that contained 4 paraffin wells were used in all germination tests (4). A drop of distilled water was placed within each of the 4 wells on a slide and chlamydospores of the organism were dispersed in the drops of water. Each slide was then placed in a Petri dish moist chamber and the chlamydospores were incubated 16 to 24 hours at room temperature.

FIELD OBSERVATIONS

Plants of *Poa pratensis* infected with *Ustilago striaeformis* were secured from pastures in Pennsylvania as early as May 5. Diseased plants were easily located from early in May until the middle of June. Later a combination of factors that included high temperature, reduced growth, and close grazing made collecting of smutted material more difficult. After the advent of hot weather, leaves infected with smut curled and withered prematurely. Premature drying of smutted plants may be severe enough to produce conspicuous patches of dead leaves. This is illustrated in figure 1, showing a clonal plot of Kentucky bluegrass with an area heavily infested with smut. Smutted plants maintained in pots both in a greenhouse and outside followed a similar course of decline. This condition continued until fall, when cooler weather once more provided an environment more favorable for growth of bluegrass.

Smutted plants of *Poa pratensis* were collected from pastures until November 15, when low temperatures prevented new growth of diseased leaves and made the plants extremely difficult to locate. Nevertheless, presence of smutted plants was demonstrated in the sods of the pastures examined. Plants in sod plugs removed from parts of pastures judged free of the disease often developed smutted shoots when the plugs were maintained at favorable temperatures in a greenhouse. When suspected diseased

plants were transplanted to a greenhouse for observation, many later developed typical smutted shoots. This suggested the possibility that smut infection among plants of *P. pratensis* may be far more extensive than is generally realized.

Many smutted plants succumb during periods of hot, dry weather. That death of smutted plants is not due to drought alone during such periods was illustrated by an observation made in the summer of 1942. More than 200 pots of smutted Kentucky bluegrass were moved from inside a greenhouse to a bed of sand outdoors. Each pot containing a diseased plant growing in soil was buried to the rim in sand. The plants were watered

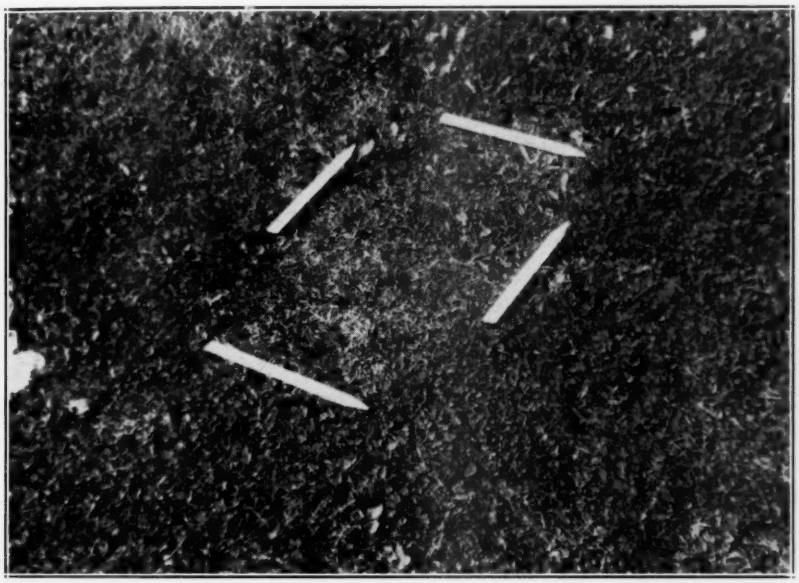

FIG. 1. Smutted plants of Kentucky bluegrass in a clonal plot in the nursery.

several times daily and shaded by a greenhouse from direct sunlight a part of each afternoon. Nearly one-half the plants died despite adequate moisture and protection from long exposure to sunlight. There also was a differential response of the plants from different collections; some continued to grow slowly throughout the summer, while others succumbed after a short time. Although the dead plants were not examined further, it seemed possible that they were so weakened by stripe smut as to be unduly predisposed to attacks by root-rotting organisms.

INFLUENCE OF DIFFERENT TEMPERATURES ON DEVELOPMENT OF DISEASED PLANTS

Smutted plants were grown at different temperatures under controlled conditions to test the relationship of this factor to the observed fluctuations in development of diseased plants. For this purpose, a refrigerated dis-

play case in a greenhouse provided the necessary temperature chambers. The display case was divided into 3 compartments each of which was designed to maintain independent, thermostatically controlled temperatures. Heating elements in each of the chambers provided the higher temperatures when desired.

Single tillers from each of 23 different smutted clones were transplanted to 4-inch pots of soil. After the young diseased plants were well established, a representative plant from each of the 23 clones was placed in the chambers at temperatures of 1.5, 10, and 32° C., and a control series of plants was kept at 20 to 25° C. in the same greenhouse that contained the temperature-control chambers. The plants were watered twice each day and were under observation during 5 months.

The smutted plants maintained at 1.5° C. for 5 months grew slowly with no visible diminution in amount of smut. Plants growing at 10° C. were vigorous and developed numerous long leaves all of which were diseased but remained green. The plants that were growing at 20 to 25° C. also were vigorous but many of the severely infected leaves turned yellow and died. Of the 23 smutted clones tested at 32° C., only 14 were alive after 4 months. Most of the leaves, present when the plants were placed at this temperature, died shortly thereafter. New leaves that emerged were long and very narrow, so that the smut spores developed within small, separated sori scattered the length of a leaf. New shoots that developed from diseased plants often produced leaves that showed no visible symptoms of smut infection.

Plants that had survived exposure to 32° C. for 4 months were removed from the temperature chamber and placed on a greenhouse bench at 20 to 25° C. At that time 9 of the 14 plants displayed no visible evidence of smut infection. After the plants had remained at the lower temperature for 1 month they were again examined for smut. Only 10 of the 14 still survived, 4 of the smutted ones having succumbed. Of the 10 remaining plants, 4 showed no evidence of smut; the other 6 bore 1 to several diseased shoots that had emerged among the smut-free tillers. When the plants were examined again after 3 months' growth at 20 to 25° C., 3 plants remained smut-free.

The results of this experiment indicate that the temperature extremes endured by the disease organism are similar to those endured by the host plant. Optimum development of the host occurred at temperatures that were favorable for growth of the smut organism. The critical maximum temperature for survival of diseased plants was approximately 32° C. This temperature was slightly less favorable for development of *Ustilago striaeformis* than for the diseased plants, since part (9 out of 14) of the treated plants recovered temporarily from all visible symptoms of smut infection. The gradual reappearance of smut in the plants that seemed to have recovered indicated probably that the fungus had remained alive in the plants but was unable to cause disease symptoms at the high temperature.

GERMINATION OF SMUT CHLAMYDOSPORES FROM PLANTS GROWING AT DIFFERENT TEMPERATURES

Davis (1) and Fischer (3), both secured germinable chlamydospores from diseased plants of *Poa pratensis* without first subjecting the spores to an after-ripening period. Since both authors worked with material collected in May, there are 2 possible explanations for their results; both authors chanced upon races of the organism that produced germinable chlamydospores or the environmental conditions that prevailed during May influenced, in some manner, the germinability of fresh chlamydospores.

In order to investigate the first possibility, germination tests were conducted on chlamydospores from diseased plants collected at different times during the growing season in widely separated pastures. Chlamydospores from more than 300 different smutted plants were examined, but none of the tests yielded more than an occasional germinated spore. The absence of germinable spores within sori on the diseased plants examined leads one to conclude that races of *Ustilago striaeformis* from *Poa pratensis* that possess germinable, fresh chlamydospores are rarely encountered, at least in central Pennsylvania.

Chlamydospores from diseased plants collected at frequent intervals from May to November showed no seasonal relationship to germinability. Additional germination tests were conducted, however, on chlamydospores from diseased plants growing at different controlled temperatures in the greenhouse. Fresh chlamydospores from plants grown at 32° C. for 3 months germinated as much as 90 per cent. Each of the surviving plants held at 32° C. yielded highly germinable chlamydospores even when the spores were removed directly from fresh green leaves. Spores from dead leaves on the same plants gave substantially lower germination percentages. Subsequent tests demonstrated that smutted plants, exposed to a temperature of 32° C. for as short a period as 2 weeks, yielded highly germinable chlamydospores.

In comparison with the results at 32° C., no germination was secured from fresh chlamydospores removed from the plants growing at 1.5, 10, and 20 to 25° C. These results indicated that the development of smut spores at relatively high temperatures, in some manner, influenced their germinability. Thus, there is the possibility that high temperatures also may influence germinability of fresh chlamydospores in the field.

EFFECT OF TEMPERATURE ON LENGTH OF AFTER-RIPENING PERIOD OF SMUT CHLAMYDOSPORES

Davis (2), earlier reported that smut chlamydospores from leaves of *Poa pratensis* required an after-ripening period of 197 days in the laboratory. Since highly germinable fresh chlamydospores were obtained from smutted plants maintained at 32° C., the possibility was considered that a similar temperature treatment might stimulate germination of chlamydospores in detached smutted leaves. Diseased leaves were, therefore, removed from

plants growing in a greenhouse at 20 to 25° C. and placed in Petri dish moist chambers in an incubator at 35° C.

In preliminary tests, excellent germination occurred 30 days after the chlamydospores were placed at 35° C. Fresh smutted leaves from a number of different clones of *Poa pratensis* were then placed in moist chambers and incubated at the same temperature. At periodic intervals, germination tests were conducted on spores from the treated leaves.

As shown in table 1, very few germinable spores were found before the incubation period was started. After five days at 35° C., most of the treated

TABLE 1.—*Per cent germination of chlamydospores of Ustilago striaeformis from Poa pratensis incubated at 35° C.*

Collection	Number of days incubated						
	0	5	10	15	20	25	30
KB14E(2)	0	0–few	few–10	25–75	30–50	0–few	
KB36G(1)	0	50–75	10	10–50	0–few	few–10	
KB34J(1)	0–few	0	25–35	0	0	10–25	
KB32A(1)	0	0–few	25–30	50	25–30	0–10	
37KB120(26)	0	0–few	25–50	75–80	30–75	few–25	
KB39K–2–7	0	few	50	30	10	10	
KB39G–2–2	0	0–few	10	75	25	75	
KB21F–3–3	0	0			65–80		
KB3C–1–3	0	few–10			80		30
KB39G–2–3	0	0			10–90		
KB31E–1–3	0	0–10			50–90		
KB39G–1–4	0	0			50		50–75
KB39L–1–3	0	0			75		80–90
KB3B–1–5	few	few–25			75–90		
KB21F–2–3	0–10	0–few			10–25		

samples yielded some germinable spores. Nearly all of the samples responded to the temperature treatment after 10 days incubation and most of the samples provided maximum numbers of germinable spores between the 10th and 20th days after treatment was started. After 20 days at 35° C., the percentage of germinating spores in each sample decreased progressively although there were appreciable numbers in some samples even after 30 days of treatment. Prolonging the incubation period beyond 30 days usually caused a pronounced decrease in percentage of germination. In tests of several collections maintained at 35° C. for 60 days the spores lost their ability to germinate despite the fact that 80 per cent of them were still germinable after 30 days.

Chlamydospores from collection KB34J(1) varied in germinability from one test to another. This is interpreted on the basis that considerable variation may exist in the state of development of chlamydospores from sori on the same or different leaves.

In a subsequent test of the effect of different temperatures, smutted leaves from each of 7 different plants of *Poa pratensis* were placed in Petri-dish moist chambers and incubated at 25, 30, 35, 40, and 45° C. At periodic intervals, germination tests were conducted on spores removed from the diseased material stored at each temperature.

Temperatures of 30 and 35° C. were found the most favorable for after-ripening the smut chlamydospores. Best germination of spores occurred when the smutted leaves were incubated at these temperatures for 10 to 30 days. There was very little germination of spores incubated at 40 and 45° C., while those incubated at 25° C. either germinated poorly or required a prolonged treatment. From the results obtained, a temperature treatment of 30 to 35° C. for 10 to 30 days seemed most favorable for after-ripening the smut chlamydospores.

GERMINATION OF SMUT CHLAMYDOSPORES IN WATER AT DIFFERENT TEMPERATURES

Davis (2) reported that chlamydospores from diseased plants of *Poa pratensis* germinated in water at a minimum temperature of 7° C. and a maximum temperature of 35° C. A temperature of 22° C. was reported as optimum. In the present investigation, the germination tests were conducted as previously described and the slides with test drops of distilled water and spores were placed at temperatures ranging from 5° to 45° C. at 5° intervals. Spores that had been treated previously at 35° C. were used. Results of the tests on spores from 3 clones are shown in table 2. The tem-

TABLE 2.—*Per cent germination of smut chlamydospores of Ustilago striaeformis from Poa pratensis in water at different temperatures*

Collection	Germination temperature								
	5° C.	10° C.	15° C.	20° C.	25° C.	30° C.	35° C.	40° C.	45° C.
KB31F–1–3	0–few	few	few–10	15–25	50–80	80	few	0	few
KB31E–1–3	0	0–few	few–10	50–70	80	80	75–80	0–few	0
37KB120(26)	0	0–few	5–10	5–10	5–35	35–85	0	0	0

peratures at which the spores germinated agree substantially with those reported by Davis. The variation among cultures with respect to the temperature at which germination occurred may possibly be attributed to differences between strains of the organism or of the host plant.

DISCUSSION

The influence of temperature on development of smutted plants was demonstrated by field observations and greenhouse experiments. The ease with which smutted plants are found at certain seasons is correlated with the temperatures that prevail at that time. Cool periods in spring and fall are most favorable for development of *Poa pratensis*. From greenhouse tests, a continuous controlled temperature of 32° C. proved maximum for survival of smutted plants. Temperatures of 1.5, 10 and 20 to 25° C. were more favorable for their growth.

When smutted plants were grown for long periods at 32° C., new emergent leaves frequently failed to display symptoms of smut infection. If

these plants were removed to a lower temperature and permitted to grow for a time, smut symptoms, with a few exceptions, again appeared. These results indicated that prolonged exposure to high temperatures was more unfavorable to the disease organism than to the host. The smut organism, however, was not completely purged from the host by this treatment; so that, for the most part, restoration of favorable environmental conditions once more permitted the smut hyphae to become established throughout the plant.

The discovery that smutted plants maintained at 32° C. yield highly germinable chlamydospores offers a possible solution to the discrepant reports in the literature regarding germinability of fresh spores of *Ustilago striaeformis*. Two authors who reported germinable chlamydospores of this organism mentioned that the collections examined were secured from the field in May. It is not unusual for temperatures to approximate 32° C. for several days at a time in the latitudes represented by these reports. Similarly, chlamydospores from smutted plants collected in other areas might escape the length of exposure to high temperatures necessary to make the spores germinable.

The fact that smutted plants of *Poa pratensis* growing at a temperature of 32° C. yield highly germinable chlamydospores does not exclude the possibility that races of the organism with germinable chlamydospores may exist. To date, however, germination tests on fresh chlamydospores from smutted plants collected in more than 75 different pastures have failed to reveal races of the organism with germinable fresh spores.

A temperature of 30 to 35° C. was found most desirable for shortening the after-ripening period of smut chlamydospores in detached smutted leaves. Usually, an after-ripening period of 10 to 30 days proved sufficient for stimulating maximum germination of the spores. Prolonged high temperature, however, apparently reduced the viability of treated spores, since a progressive decrease in numbers of germinable spores resulted when the samples were incubated more than 30 days.

Chlamydospores, incubated at temperatures below 30° C., required a considerably longer period to after-ripen, while spores incubated at temperatures above 35° C. failed to germinate, even after prolonged treatment. The results of the experiments reported herein demonstrate that temperature is a prime factor in determining the length of time necessary to after-ripen chlamydospores of *Ustilago striaeformis* from *Poa pratensis*.

SUMMARY

High temperatures in the field and in greenhouse experiments were unfavorable for development of *Poa pratensis* infected with *Ustilago striaeformis*.

Part of the smutted plants exposed to a continuous temperature of 32° C. lost their smut symptoms after 4 months, but, with a few exceptions, regained them when the plants were grown at a lower temperature.

Chlamydospores removed from leaves of smutted plants growing at 32° C. proved highly germinable, without any after-ripening period.

The after-ripening period of chlamydospores of *Ustilago striaeformis* was reduced from 200 days to less than 30 days by incubating detached smutted leaves in a moist chamber at 35° C.

Examination of chlamydospores from more than 300 smutted plants of *Poa pratensis* collected in 75 different pastures failed to reveal the presence of races of the organism with germinable fresh spores.

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NEW AND STANDARD SEED TREATMENTS IN THE CONTROL OF CERTAIN SEED-BORNE DISEASES OF WHEAT, OATS, AND BARLEY[1]

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During the 1942 season, cooperative tests were conducted at several stations in Canada to determine the value of certain new and standard fungicidal seed-treatment materials for the control of smut (*Ustilago avenae* (Pers.) Jens. and *U. levis* (Kell. and Sw.) Magn.) in oats, covered smut (*Ustilago hordei* (Pers.) Lagerh.) in barley, seedling blight (*Helminthosporium sativum* P. K. and B.) in wheat and barley, and leaf blotch (*Helminthosporium avenae* Eidam) in oats.

MATERIALS AND METHODS

The following dry disinfectants were used: Ceresan, 5 per cent ethyl mercuric phosphate (Bayer-Semesan Co.); "Half Ounce" Leytosan, 7.2 per cent phenyl mercury (F. W. Berk & Co., Ltd., London, England); Spergon, 99 per cent tetrachloro parabenzoquinone (U. S. Rubber Co.); N.G. 3399/9 K, an unnamed dust (Canadian Industries Ltd.); Thiosan (DuBay 1205–FF), 50 per cent tetra-methyl thiuramdisulphide (Bayer-Semesan Co.); Chemical No. 601 and Chemical No. 604 (U. S. Rubber Co.). Ceresan and Leytosan were applied at the rate of $\frac{1}{2}$ oz. per bu. of grain, Spergon and N.G. 3399/9 K dust at 3 oz. per bu., and Thiosan, Chemical No. 601, and Chemical No. 604 at 2 oz. per bu.

In addition to the dry disinfectants mentioned above, the following materials were used as wet treatments: Formaldehyde, Ceresan, and Bordeaux mixture. Formaldehyde was used at the standard rate of one part of Formalin (40 per cent formaldehyde to 320 parts of water). The Ceresan solution consisted of 1 part of Ceresan to 800 parts of water, by weight. The grain was immersed in the formaldehyde and Ceresan solutions for 3 minutes. After treatment it was covered for 4 hours, then dried and placed in small paper envelopes ready for seeding. The Bordeaux mixture used was prepared by dissolving 20 g. of powdered $CuSO_4$ and 20 g. of $Ca(OH)_2$ in 1000 cc. of water. The seed was immersed in this solution for 15 minutes, dried, and packaged. All seed treatments were made from 15 to 30 days prior to seeding time.

Severely naturally infected seed from the crops of 1941 was used in all experiments. The smut tests were made with Anthony oats and Trebi barley. The oat seed carried a smut spore load equivalent to one part of

[1] Experiments carried out in cooperation with the Officers in Charge of the Dominion Laboratories of Plant Pathology at Edmonton, Alta., Saskatoon, Sask., St. Catharines, Ont., Ottawa, Ont., Fredericton, N. B., Kentville, N. S., and Charlottetown, P. E. I., and with Prof. J. G. Coulson, Macdonald College, Quebec. Contribution No. 736, Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

spores (mostly *Ustilago avenae*) to 16,000 parts of seed, by weight, and the barley a spore load of 1:3,500. Seed lots of Charlottetown 80 barley and Reward wheat with 86 per cent and 52 per cent of the kernels, respectively, internally infected with *Helminthosporium sativum*, were used in the seedling-blight tests. Erban oats was used in the oat-leaf-blotch experiments. An agar-plate test of this seed showed that 38 per cent of the kernels was internally infected with *Helminthosporium avenae*. The treated seed, along with similarly diseased nontreated seed of Anthony oats, Trebi barley, Charlottetown 80 barley, and Erban oats, was planted at Winnipeg, Manitoba, in four separate experiments at the rate of 200 seeds per 8-foot row. The system of replication used in each experiment consisted of 4 randomized blocks of 8 rows each. Similar plantings were made at 8 other stations in Western Canada, and at 7 stations in Eastern Canada. The results of these experiments are summarized in tables 1 and 2.

In addition to the above-mentioned cooperative tests, greenhouse and field experiments were made at Winnipeg to determine the effect of certain standard and new fungicidal seed treatment materials, including Spergon and Thiosan, on the control of seedling blight in wheat and barley and of leaf blotch in oats, and on plant emergence and yield. The field experiments were repeated at Morden, Manitoba. In the greenhouse tests, 100 seeds of each kind of treated and untreated seed of Reward wheat, Charlottetown 80 barley, and Erban oats were planted in rows in large beds of non-sterile soil, maintained in proper condition, and in which the amount of soil-borne infection was negligible. Data on emergence and disease control were obtained 15 days after the seed had been sown. The greenhouse tests were run at two different times. Field experiments, similar to those carried out in the cooperative tests, were made at Winnipeg and Morden, Man. In these tests, treated and untreated diseased seed of Reward wheat as well as Charlottetown 80 barley and Erban oats was planted in separate experiments. The materials and rates of application used are given in tables 3 and 4.

Three separate experiments were made at Winnipeg and Morden to determine the effect of different seed treatments on the yield of diseased seed of Reward wheat, Charlottetown 80 barley, and Erban oats. The treated seed, along with untreated seed of each variety, was planted in rod rows at the rate of from 400 to 500 seeds per row, depending upon the variety, replicated 6 times for each fungicide. Thus, each yield test consisted of 6 randomized blocks of 10 rod rows each. Yields were finally computed on the basis of bushels per acre. The results of the yield tests are presented in table 4.

Data on emergence in all field experiments were obtained about 30 days after planting; those for the greenhouse tests, when the seedlings were 15 days old. The disease data of the smut experiments (Table 1) were recorded at or near harvest time. The total number of heads and number of smutted heads per 8-foot row were counted; and the percentage of smutted heads

per row was computed. The method of recording the disease data in the cooperative barley seedling blight and oat leaf blotch tests (Table 2), and in the special wheat and barley seedling blight and oat blotch experiments (Tables 3 and 4), was as follows: Immediately after the emergence data had been recorded, the young plants were lifted from the soil, cleaned, and examined individually for presence of basal or leaf lesions caused by seed-borne fungi, and the percentage of diseased plants per row was determined.

The emergence, disease, and yield data of each field experiment, with the exception of the disease data of the smut experiments, were treated statistically by the analysis of variance method.

EXPERIMENTAL RESULTS

The results of the oat- and barley-smut experiments are given in table 1. These data show that Ceresan and Leytosan gave perfect control of smut in oats, and almost perfect control of covered smut of barley, while the Ceresan-dip and formalin-dip treatments were only slightly less effective. Spergon and Bordeaux mixture gave only partial smut control. N.G. 3399/9 K dust was quite ineffective in controlling the smuts of oats and barley. The formalin dip treatment reduced emergence in oats, and Ceresan dip, in barley.

TABLE 1.—*Effect of seed treatment with different fungicides on seedling emergence, and on the occurrence of smut in oats, and covered smut in barley. Results of cooperative cereal seed treatment experiments at stations in eastern and western Canada in 1942*

Fungicide	Oz. per bu.	Oat smut[b] (*U. avenae* and *U. levis*)				Barley smut[c] (*Ustilago hordei*)			
		Mean percentage emergence (Emer.) and mean percentage heads smutted (Smut)							
		Eastern, 7 stations		Western, 9 stations		Eastern, 6 stations		Western, 9 stations	
		Emer.	Smut	Emer.	Smut	Emer.	Smut	Emer.	Smut
Ceresan	½	78	0.0	84	0.0	84	0.1	87	0.01
Leytosan	½	77	0.0	83	0.0	82	0.2	87	0.02
Spergon	3	80	2.6	81	0.9	84	0.9	88	0.6
N.G. 3399/9 K	2	77	3.8	77	1.9	81	1.1	86	1.0
Ceresan dip	.....	78	0.01	84	0.2	70	0.01	75	0.02
Formalin dip	.....	71	0.01	74	0.01	82	0.9	89	0.4
Bordeaux mixture	.....	76	1.4	79	0.8	80	1.1	83	0.9
None	.....	78	7.1	78	2.7	85	4.0	87	4.1
Necessary difference, 5% level	.....	4.3	[a]	4.0	[a]	4.1	[a]	3.7	[a]

[a] Smut data not analyzed statistically.
[b] Variety = Anthony. Smut spore load on seed = 1 : 16,000.
[c] Variety = Trebi. Smut spore load on seed = 1 : 3,500.

The amount of smut observed in the untreated oats at the different stations ranged from 1.3 to 11.2 per cent, with an average of 7.1 per cent for 7 stations in Eastern Canada and 2.7 per cent for 9 stations in Western

Canada. Smut in the untreated barley ranged from 1.1 to 16.4 per cent, and averaged only 4.0 per cent for the 15 stations in Eastern and Western Canada. Obviously, the fungicides were not subjected to a very severe test in 1942. However, owing to the fact that exceedingly uniform smut control results were obtained with each fungicide at 15 widely separated stations, and also to the fact that almost perfect control of smut was obtained at all stations with the organic mercury compounds, Ceresan and Leytosan, it is considered that the results of the 1942 tests give a fairly good indication of the possible effectiveness of the fungicides tested in controlling covered smut of barley and smut in oats.

TABLE 2.—*Effect of seed treatment with different fungicides on seedling emergence, and on the occurrence of seedling blight (Helminthosporium sativum) in barley, and leaf blotch (Helminthosporium avenae) in oats. Results of cooperative cereal seed treatment experiments at stations in eastern and western Canada in 1942*

Fungicide	Oz. per bu.	Seedling blight of barley[a] (*Helminthosporium sativum*)				Leaf blotch of oats[b] (*Helminthosporium avenae*)			
		Mean percentage emergence (Emer.) and mean percentage seedlings diseased (Dis.)							
		Eastern, 5 stations		Western, 9 stations		Eastern, 6 stations		Western, 6 stations	
		Emer.	Dis.	Emer.	Dis.	Emer.	Dis.	Emer.	Dis.
Ceresan	½	84	3.5	89	11.6	78	16.4	90	3.0
Leytosan	½	84	2.7	90	11.3	78	23.6	89	4.3
Spergon	3	62	20.5	66	74.6	74	42.4	86	20.5
N.G. 3399/9 K	2	70	26.2	75	77.9	78	33.9	90	26.2
Ceresan dip	.....	82	3.0	89	10.6	74	23.8	84	4.3
Formalin dip	.....	77	20.0	80	67.6	76	33.6	85	20.0
Bordeaux mixture	.....	63	25.5	68	82.2	76	43.3	83	25.5
None	.....	63	25.9	70	78.9	75	39.7	80	25.9
Necessary difference, 5% level	.....	4.5	13.5	4.1	6.6	[c]	10.1	4.6	11.2

[a] Variety = Charlottetown 80. Percentage of seeds infected = 86 per cent.
[b] Variety = Erban. Percentage of seeds infected = 38 per cent.
[c] Differences between fungicides insignificant statistically.

The results of greenhouse and field experiments designed to determine the effect of different seed treatments on the control of seedling blight (*Helminthosporium sativum*) in wheat and barley, and leaf blotch (*H. avenae*) in oats, and on plant emergence and yield, are given in tables 2, 3, and 4. From these data it is evident that, on the whole, the organic mercury seed-treatment materials, Ceresan, Leytosan, and Ceresan dip, were highly effective in controlling seed-borne diseases of cereals caused by species of *Helminthosporium*, whereas Spergon and Thiosan were quite ineffective. Although Chemical No. 604 gave fairly effective control of leaf blotch of oats and partial control of seedling blight in wheat, it was ineffective in controlling barley seedling blight (Tables 3 and 4). Treatment of severely naturally infected seed of wheat, oats, and barley with Formalin dip, Chem-

ical No. 601, Bordeaux mixture, and N.G. 3399/9 K dust, did not reduce the incidence of seedling blight in either wheat or barley, or of leaf blotch in oats. The results obtained in beds of ordinary soil in the greenhouse agreed very closely with those obtained in the field.

TABLE 3.—*Effect of seed treatment with different fungicides on the control of seedling blight in wheat and barley and leaf blotch of oats, and on seedling emergence. Results of greenhouse soil-bed tests. (Data are means of 2 trials)*

Fungicide	Oz. per bu.	Seedling blight of wheat[a] (*H. sativum*)		Seedling blight of barley[b] (*H. sativum*)		Leaf blotch of oats[c] (*H. avenae*)	
		Percentage seedling emergence	Percentage seedlings diseased	Percentage seedling emergence	Percentage seedlings diseased	Percentage seedling emergence	Percentage seedlings diseased
Ceresan	½	98	0	98	0	95	0
Leytosan	½	96	0	99	1	96	0
Spergon	3	92	32	89	66	94	18
Chemical #601	2	75	49	90	44	94	16
Chemical #604	2	94	18	96	36	95	10
Thiosan	2	97	21	90	33	92	9
N.G. 3399/9 K	3	78	25	89	50	92	11
Ceresan dip	.....	98	0	96	0	94	0
Bordeaux mixture	.....	87	48	86	56	95	18
None	.....	70	46	86	50	93	14

[a] Variety = Reward. Percentage of seeds infected = 52 per cent.
[b] Variety = Charlottetown 80. Percentage of seeds infected = 86 per cent.
[c] Variety = Erban. Percentage of seeds infected = 38 per cent.

The field results in table 4 show that Ceresan, Leytosan, Spergon, Thiosan, N.G. 3399/9 K dust, and Chemical No. 604, were consistently beneficial to emergence, while Bordeaux mixture, and, in general, Chemical No. 601, were of little benefit in this respect. As might be expected from the disease control results, highly significant increases in yield were obtained over nontreated seed when wheat seed severely infected with *Helminthosporium sativum* was treated with Ceresan or Leytosan. Most of the other fungicides also increased yield significantly. In general, wheat yield was increased in direct proportion to the amount of seedling blight control. Unfortunately, individual plot yields of the oat and barley experiments varied widely in 1942, and hence the yield differences observed between seed treatments in these tests were, for the most part, statistically insignificant.

The results presented in tables 1, 2, 3, and 4 show the marked effectiveness of the organic mercury seed disinfectants, Ceresan and Leytosan, in controlling certain destructive seed-borne diseases of wheat, oats, and barley. In comparison with these standard fungicides, the new materials, Spergon, Thiosan, Chemical No. 601, and Chemical No. 604, were quite ineffective. Spergon is on the market as a vegetable seed protectant and Thiosan as a fungicide for turf. Chemical No. 601 and Chemical No. 604 are still in the experimental stage. In 1942, Spergon and Thiosan were quite unsatis-

TABLE 4.—*Effect of seed treatment with different fungicides on the occurrence of seedling blight in wheat and barley, and of leaf blotch in oats, and on seedling emergence and yield. Results of field experiments at Winnipeg and Morden, Manitoba, in 1942*

Fungicide	Oz. per bu.	Winnipeg			Morden		
		Seedling blight of wheat[b] (*Helminthosporium sativum*)					
		Percentage seedling emergence	Percentage seedlings diseased	Yield (bu. per ac.)	Percentage seedling emergence	Percentage seedlings diseased	Yield (bu. per ac.)
Ceresan	½	74	18.5	53.6	92	15.1	42.9
Leytosan	½	75	17.4	54.1	94	15.6	41.2
Spergon	3	71	48.6	41.6	68	49.4	32.6
Chemical #601	2	49	60.5	34.2	41	59.9	23.8
Chemical #604	2	79	40.7	50.1	80	48.3	37.7
Thiosan	2	74	50.8	48.3	77	51.2	37.1
N.G. 3399/9 K	3	62	55.9	44.2	63	60.8	30.3
Ceresan dip	.....	82	19.1	51.8	92	22.8	36.2
Bordeaux mixture	.....	56	57.4	37.8	44	58.8	27.2
None	.....	40	60.4	28.2	23	56.4	19.4
Necessary difference, 5% level	.....	10.5	13.2	2.8	7.4	8.8	5.1
		Seedling blight of barley[c] (*Helminthosporium sativum*)					
Ceresan	½	91	18.3	39.4	91	18.6	53.8
Leytosan	½	87	22.7	37.1	92	23.3	54.4
Spergon	3	72	58.4	38.6	73	73.8	51.5
Chemical #601	2	78	57.3	41.7	74	92.9	49.6
Chemical #604	2	76	58.9	38.1	80	77.8	53.7
Thiosan	2	77	48.9	38.1	78	86.6	57.7
N.G. 3399/9 K	3	79	59.1	37.0	74	88.8	57.3
Ceresan dip	.....	85	16.2	35.7	88	18.8	48.1
Bordeaux mixture	.....	69	66.0	35.6	66	90.7	42.6
None	.....	67	62.3	31.3	72	91.5	45.7
Necessary difference, 5% level	.....	8.6	6.8	[a]	5.8	7.2	9.1
		Leaf blotch of oats[d] (*Helminthosporium avenae*)					
Ceresan	½	86	2.6	58.0	92	2.4	95.5
Leytosan	½	86	1.9	62.6	93	2.1	93.8
Spergon	3	80	57.0	62.5	80	42.9	84.6
Chemical #601	2	81	55.0	53.9	82	48.8	91.4
Chemical #604	2	80	33.9	59.5	86	23.2	97.4
Thiosan	2	82	44.9	60.7	89	33.0	88.0
N.G. 3399/9 K	3	73	31.0	60.4	88	33.5	90.0
Ceresan dip	.....	79	1.9	65.1	91	2.4	98.5
Bordeaux mixture	.....	74	49.3	60.4	86	42.8	82.7
None	.....	75	57.2	61.4	82	55.5	82.8
Necessary difference, 5% level	.....	[a]	9.8	[a]	4.1	8.6	[a]

[a] Differences between fungicides insignificant statistically.
[b] Variety = Reward wheat. Percentage of seeds infected = 52 per cent.
[c] Variety = Charlottetown 80. Percentage of seeds infected = 86 per cent.
[d] Variety = Erban. Percentage of seeds infected = 38 per cent.

factory for the control of seedling blight of wheat and barley, and leaf blotch of oats, but further tests with these new non-metallic fungicides against these and other destructive seed-borne diseases of cereals would be desirable.

SUMMARY

Field experiments were made in 1942 to determine the effect of certain standard and new fungicidal seed treatment materials on the control of smut (*Ustilago avenae* and *U. levis*) in oats, covered smut (*Ustilago hordei*) in barley, seedling blight (*Helminthosporium sativum*) in wheat and barley, and leaf blotch (*Helminthosporium avenae*) in oats. Severely naturally infected seed was used in all experiments.

The organic mercury seed disinfectants, Ceresan, Leytosan, and Ceresan dip, gave excellent control of smut in oats, covered smut of barley, seedling blight of wheat and barley, and leaf blotch of oats. Formalin dip controlled smut effectively, but was quite ineffective in controlling barley seedling blight and oat leaf blotch. In 1942, the new synthetic organic chemicals, Spergon and Thiosan, gave little promise as cereal seed disinfectants.

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A NEW METHOD FOR COMPUTING SUGAR BEET LEAF AREA[1]

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(Accepted for publication March 29, 1943)

Many agricultural research workers have interested themselves in the past few years with finding the areas of leaves of various plants. Gustafson and Stoldt (5) studied the relation between leaf area and fruit size in tomatoes. Haller and Magness (6) investigated the association of leaf area and the growth and composition of apples, and Schuster (16) carried out experiments for determining the influence of leaf area and shoot growth in relation to size and filling of filberts. Davis (2) developed a formula for finding areas of bean leaves, and Porter[4] found a parabolic formula for areas of tomato leaves. Magness and Overley (10, 11) investigated the effect of leaf area upon size and quality of apples and pears, and Overholser and Claypool (14) carried out research on the relation of leaf area per peach to physical properties and chemical composition. Swanson (17) studied the influence of leaf area of sorghum versus yield. Mitchell (13), Frear (3), Gerdel and Salter (4), Kramer (9), Withrow (18), Hibbard, Grigsby and Keck (8) developed photo-electric devices for the measurement of leaf areas and Marshall (12) constructed an apparatus for obtaining the areas of raspberry leaves. Baten constructed a nomogram for finding the area of bean leaves (1).

There are several experiments now being carried out at Michigan State College in which areas of leaves form important parts of the investigations; these pertain to counts of European red mites per leaf area of Delicious apple tree leaves and effectiveness of sprays; effects of sprays in relation to transpiration of sugar-beet leaves and honeysuckle leaves; leaf area versus yield of field beans; etc.

In experiments designed to determine the effect of spray or dust materials upon transpiration rate of plants, it is necessary to employ an accurate and rapid method of finding the leaf area. The standard method of finding leaf area is by means of the planimeter. Although this method is accurate, it involves a great amount of tedious, painstaking effort and consumption of valuable time.

In recent experiments designed to determine the effect of copper sprays and dusts upon the transpiration rate of sugar beet leaves in the greenhouse it became highly desirable to employ some method other than planimeter readings for determining leaf area. Sugar-beet leaves of varying ages were

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[4] Porter, A. M. The influence of defoliation and fruit thinning on the growth of tomatoes. Unpublished thesis, Michigan State College, 1932.

cut from the plant, and dried between blotters in a plant press. The ruffled leaf margins were smoothed as much as possible before the leaves were completely dried. After drying, planimeter readings were made of the area of 200 leaves. These were then used in checking against the data obtained by using a new method derived by the writers.

Areas of the leaves, obtained by the planimeter, were plotted against the widths, the lengths, widths and lengths, and also against the products of the widths and lengths. Straight lines were found by the method of least squares, for predicting leaf areas from these respective measurements. The standard errors of estimate, which indicate how accurate predictions are,

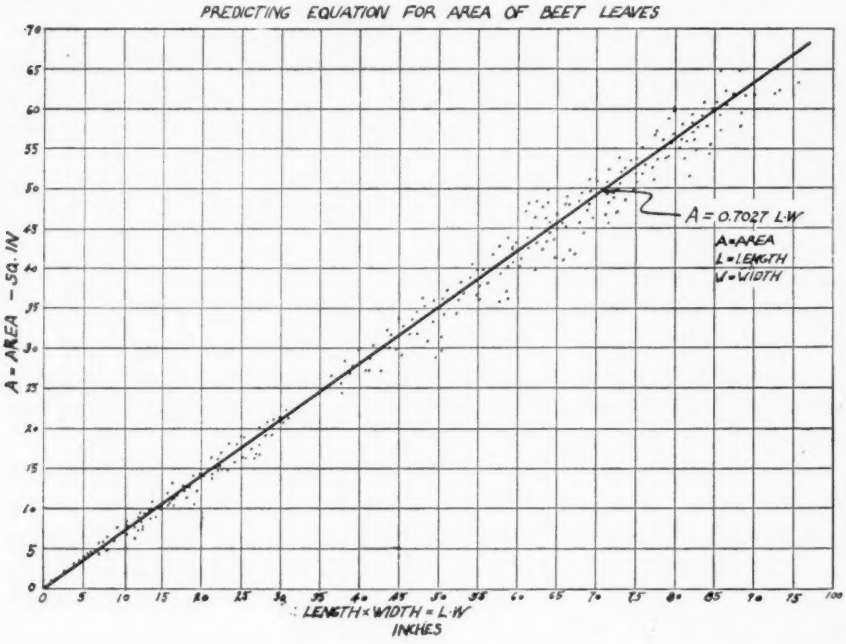

FIG. 1.

were found in each case, together with correlation coefficients. The smallest standard error of estimate was found where the product of width and length was used for predicting leaf area. The predicting equations pertaining to leaf area and product of length and width are as follows:

Small leaves—1941: $A = 0.6974P$,
Large leaves—1942: $A = 0.7035P$,
All leaves: $A = 0.7027P$,

where A represents leaf area and P represents the product of width and length. These equations without a constant term were about as reliable as the equations with such a term; these equations with a constant term are not given. Since the equation for predicting areas for small leaves differs very little from that for predicting areas for large leaves, all of the data were combined for obtaining one predicting equation. This equation is

$$A = 0.7027P;$$

and it was used in constructing the nomogram. Figure 1 shows the scatter diagram about this straight line.

The nomogram was obtained by using the linear relation between the logarithms of the area, 0.7027L and W as

$$\text{Log } A = \text{Log } 0.7027L + \text{Log } W.$$

The scales on each of the axes, shown in figure 2, were obtained as logarithmic scales for the chosen interval of 12 inches for the heights of the axes of the measurements pertaining to width and length measurements. Methods for constructing nomograms are given in any text on nomography (7, 15).

The area of a sugar-beet leaf with width and length equal, respectively, to 3.4 in. and 4.5 in. is found by laying a straight edge on the width axis (Nomogram, Fig. 2) on 3.4 and on the length axis on 4.5 and then reading the area of the leaf on the area axis; this is found to be 10.8 sq. in. The area of a sugar-beet leaf with width and length respectively equal to 7.6 in. and 9.2 inches is found by laying the straight edge on the width axis on 7.6 and the length axis on 9.2 and then reading the area on the area axis, where the straight edge crosses this axis; this is 49.1 sq. in. By using the nomogram, one can find the areas of the leaves in the field or in the greenhouse without removing them from the plants and without any computations. One person can measure the width and length of the leaf on the plant, while a second person reads from the nomogram, for these measurements, the area of the leaf. It is necessary only to record one value, the area of the leaf. In this way 2 people can determine the areas of many leaves in a relatively short time. By this method the plants are not mutilated.

The nomogram is of little value unless one is going to measure many leaves or is going to carry on the study with the same kind of leaves at least 2 years, or when the equation for finding leaf area in terms of length and width measurements does not hold from season to season. However, if the investigation is going to extend over several years, as many do, and the equation obtained from measurements the first years is suitable for the measurements of the same kind of leaves the succeeding years, as often happens, then the nomogram constructed from the first-year measurements will be beneficial in all future work. Davis (2) has used an equation for the area of bean leaves several seasons; in his case the nomogram will greatly reduce the amount of time and labor devoted to finding leaf areas. Many people use the information gathered the first year or for the first four or five hundred leaves for determining the areas of all future leaves; in these cases it would pay to construct a nomogram from the first set of data and use it for future work.

This article was written for the purpose of calling attention to this very handy method of finding the area of leaves. Similar methods might be used for other relations. The nomogram for obtaining areas of beet leaves will not be applicable for other kinds of leaves.

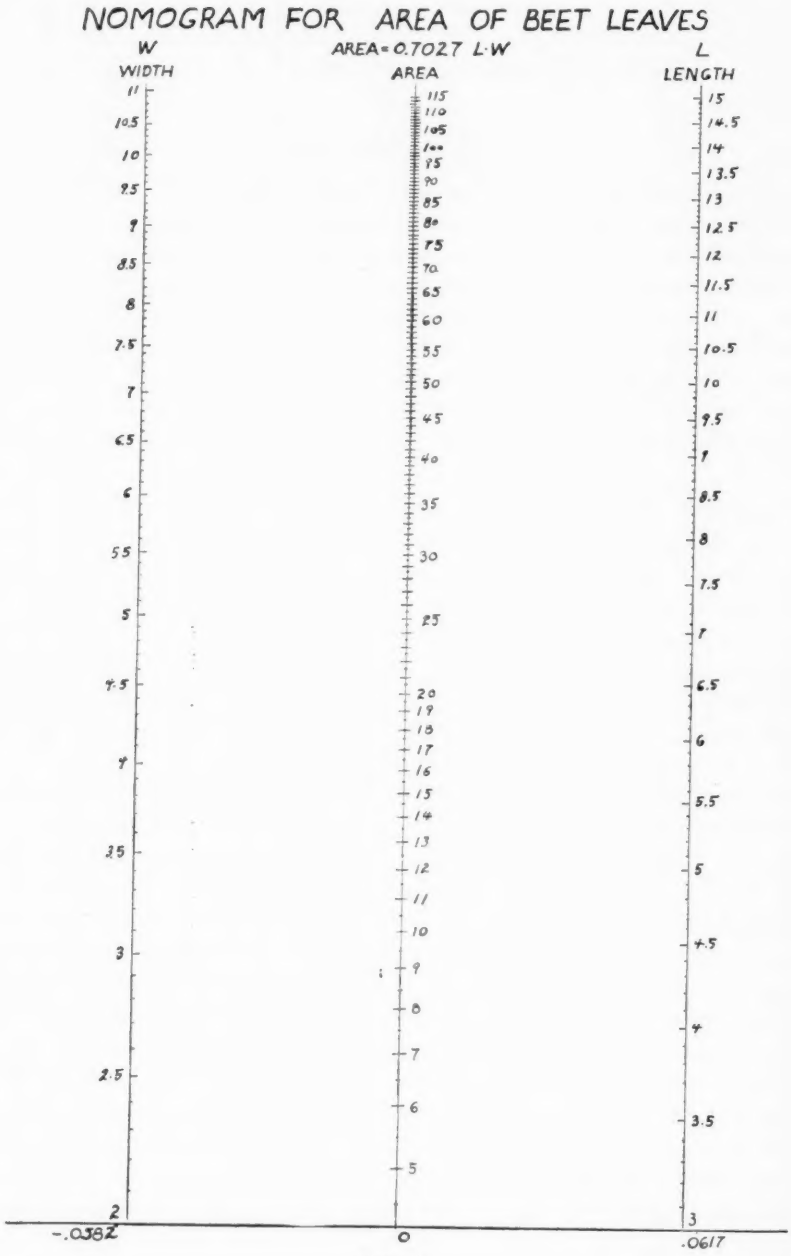
NOMOGRAM FOR AREA OF BEET LEAVES
AREA = 0.7027 L·W
W
WIDTH
AREA
L
LENGTH
-.0382
0
.0617

FIG. 2.

SUMMARY

A method has been devised for rapid determination of areas of sugar-beet leaves. For this purpose a nomogram was obtained by using the linear relation between the logarithms of the area, the length and the width of the leaves.

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DISTRIBUTION OF BACTERIAL WILT (BACTERIUM SOLANACEARUM) IN SUCCESSIVE CROPS OF TOBACCO GROWN ON THE SAME FIELDS[1]

T. E. SMITH[2]

(Accepted for publication March 30, 1943)

INTRODUCTION

Bacterial wilt occurs in spots in many tobacco fields. In some areas 50 to 100 per cent of the plants may be affected, while in other areas of the same field one per cent or less may be diseased (Fig. 1).

Whether or not these patterns of distribution remain constant or change from season to season was of importance in studying control measures. A marked increase in wilt severity in an area of the field where it was present only in small amount or absent on the previous tobacco crop would indicate that the pathogen had been spread by surface water or other means. Therefore, cultural practices that reduced rapidity of spread would be of value in controlling the disease. Information was not available on the stability of the pattern of occurrence and to study this problem maps were made of wilt distribution during two or more successive crops of tobacco.

METHODS

In the fields selected for study wilt had been present in varying amount for perhaps 20 to 30 years. According to the farmers each field had been wholly planted to tobacco or other crops in 1936 and 1937. The survey was undertaken in 1938 by dividing the fields into plots of 50×50 ft., an area sufficient for approximately 250 tobacco plants. Wilt counts were made during the first few days of August. Concurrently, the elevation of each plot above the lowest point in the field was determined with a transit. Plots were laid out and data taken on a total area of 11.9 acres in 7 fields. All plots, except 3, contained some wilted tobacco plants in 1938. Four of the fields were planted to tobacco in 1940 or 1942, depending on the length of rotation used, and at that time the plots on these fields were relocated for additional wilt counts. Early-season incidence was studied on 2 fields in counts made June 10, about 5 weeks after transplanting. Final distribution of the disease was studied in counts made August 1 to 15, on completion of harvesting. The number of plots, average amount of wilt in 1938, rotation used, and average amount of wilt on the succeeding crop of tobacco are

[1] Cooperative investigations of the Division of Tobacco Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, the N. C. Department of Agriculture, and the N. C. Agricultural Experiment Station. Published with the approval of the Director as paper No. 161 of the Journal series.

[2] The writer expresses appreciation to R. K. Godfrey and J. F. Gilmore for assistance in the field work and to the Department of Experimental-Statistics of the Experiment Station for assistance in the statistical computations.

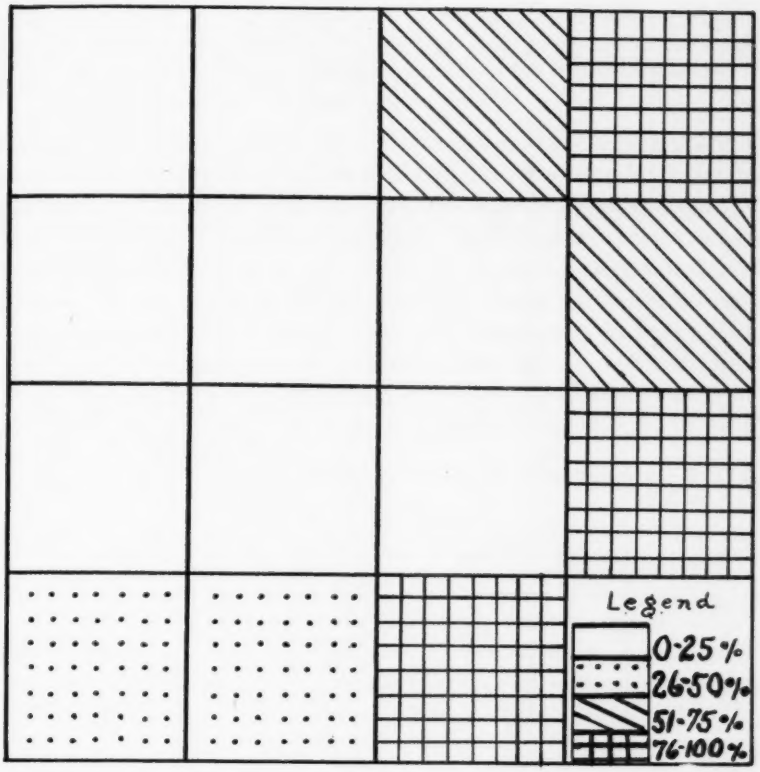

FIG. 1. Distribution of bacterial wilt in a tobacco field. Each square represents an area of 50 × 50 feet with the indicated amount of wilt.

TABLE 1.—*Number of plots, average amount of wilt and rotations used in the fields studied*

Field	Plots	Amount of wilt on tobacco grown in 1938	Rotation used	Amount of wilt on tobacco grown in 1940 or 1942
	Number	*Per cent*	*Year and crop grown*	*Per cent*
1	32	7 ± 9[a]	1939, crab grass	
			1940, tobacco	11 ± 11
			1941, corn	
			1942, tobacco	25 ± 22
2	12	42 ± 33	1939, lespedeza	
			1940, tobacco	52 ± 33
3	15	38 ± 30	1939, corn	
			1940, tobacco	71 ± 21
4	50	63 ± 22	1939, wheat and lespedeza	
			1940, lespedeza	
			1941, corn	
			1942, tobacco	85 ± 12
5	31	14 ± 12		
6	31	62 ± 17		
7	37	53 ± 19		

[a] Mean and standard deviation.

shown in table 1. The amount of wilt varied widely between fields and within fields, as shown by these data.

RELATION OF WILT SEVERITY IN 1938 OR 1940 TO THAT OF SUCCEEDING CROP

The first problem studied was the relation of wilt severity in 1938 to the areas of the field where wilt first occurred in the tobacco crop the next year. Table 2 presents the data on a field of 12 plots that, for convenience, have been arranged on the basis of the amount of wilt in each plot in 1938. Wilt varied among the different plots from 6 to 96 per cent at the end of the season in 1938. In 1940, 5 weeks after the tobacco was set, some wilt was present on nearly all plots, but the highest per cent occurred on the plots with the most wilt in 1938. The amount of wilt at the end of the season in 1940 was closely related to the early-season incidence. Data on another field of 32 plots were similar to those reported in table 2.

TABLE 2.—*Relation of wilt distribution in 1938 to early season incidence and distribution in 1940*

Plot	Amount of wilt during the stated years		
	1938	1940	
	At end of season	Five weeks after transplanting	At end of season
Number	*Per cent*	*Per cent*	*Per cent*
6	6	2	11
2	9	2	20
10	15	2	23
5	15	2	27
1	20	0	24
9	26	2	38
7	37	7	42
3	48	9	60
11	51	10	55
8	82	10	97
4	96	16	99
12	96	26	99

The relationship between pattern of occurrence of wilt in successive tobacco crops was determined by means of correlation coefficients. In field numbers 1, 2, 3, and 4 (Table 1), data were available on the percentage of wilted plants on each plot in 1938 and in 1940 or 1942, depending on the length of rotation used. The values of "r" between the amount of wilt present in 1938 and on the next tobacco crop were 0.9645**,[3] 0.9307**, 0.8011** and 0.7115**. Field number 1 was planted in tobacco during 1938, 1940, and 1942. The multiple correlation coefficient (R) between percentage of plants wilted in 1938 (x_1), percentage of plants wilted in 1940 (x_2) and percentage of plants wilted in 1942 (y) was 0.9052**. The over-all correlation "r" for the entire set of data on 141 plots between per-

[3] ** Highly significant.

centage of wilted plants in 1938 or 1940 and percentage of wilted plants in the next tobacco crop was 0.9036**. All of these coefficients are quite high, showing a close relationship between the pattern of wilt distribution on tobacco grown during different seasons.

RELATION OF ELEVATION TO WILT SEVERITY

The relation between elevation and wilt severity was studied in ten sets of data, using wilt counts made in 1938, 1940 and 1942. Correlation coefficients (r) were computed between elevation above the lowest point in the field and the percentage of wilted tobacco plants. These were 0.3317*,[4] 0.2583, 0.1895, −0.0444, −0.2183, −0.3271, −0.3512*, −0.4438, −0.8845**, −0.9053**. The coefficients were highly variable, ranging from positive to negative with statistically significant values on both sides of zero. However, the negative relationship was found in seven of the ten sets of data. Hence, in most fields wilt was more severe in the lower-lying areas but this relationship was not consistent for all fields.

DISCUSSION

The factors that might explain the wide differences in amount of wilt in different parts of a field may, for purposes of discussion, be divided into 2 groups. The first group includes factors that change from season to season, such as the chance introduction of inoculum into the field, spread of inoculum within the field from sites where it had overwintered in the soil, biological effects that might change disease severity and others. If wilt distribution were largely dependent on any or all of these factors, the pattern of distribution would change from year to year. No evidence of a major change in wilt distribution was found; therefore, it is doubtful that such seasonal factors governed the amount of wilt in different areas of the fields studied. The second group of factors determining wilt distribution includes the relatively stable characteristics of the soil and physical features of the field that might effect pathogen or host. Several writers, van der Meer[5] and others, have pointed out that bacterial wilt was more severe on soils of high moisture content. This factor was associated with wilt distribution in some fields. For example, one end of field No. 2 bordered a poorly drained woodland, and inspection showed that soil moisture was considerably higher in the plots in that part of the field. The highest percentages of wilted plants also occurred in the plots nearest the poorly drained woodland, indicating a relationship with soil moisture. However, in other fields, with what appeared to be well-drained soil throughout, variations in amount of wilt also occurred, showing that factors other than proximity of the water table played a part in determining wilt distribution. Bacterial wilt is water-borne and it was expected that repeated movement of contaminated

[4] * Significant.

[5] van der Meer, J. H. H. The influence of degree of soil humidity on slime disease caused by *Bacterium solanacearum*. Bull. Deli Proefsta. (Medan, Sumatra) 29, 1929. (In Dutch with English summary, pp. 36 to 49.)

soil by surface water would cause uniformly severe wilt damage in the lower-lying plots. But, as pointed out previously, the correlation between elevation and amount of wilt varied widely on different fields, showing that direction of flow of surface water was not uniformly associated with wilt distribution. The over-all correlation coefficient of 0.9036** between the amount of wilt in the preceding crop and the succeeding crop suggested that wilt severity was mainly determined by the location of the plot in the field. It appeared that bacterial wilt was in equilibrium with the more stable chemical and physical properties of the soil.

The problems involved in control of bacterial wilt by crop rotation were discussed in an earlier publication.[6] It was shown that the pathogen was not starved out by as much as 4 years of bare fallow. It also was shown that elimination of wilt-susceptible weeds from the immune crop did not increase the degree of control obtained from the rotation. These results illustrate the difficulties of controlling the disease by crop rotation. The data reported here are related to control of the disease by methods of sanitation. Bacterial wilt recurs in proportion to the amount present in the previous tobacco crop. Hence, in fields where wilt has been present for several years, cultural practices tending to reduce spread appear to be of negligible value.

SUMMARY

Bacterial wilt tends to be unevenly distributed in many tobacco fields. Information was lacking as to whether the pattern of occurrence was similar in successive crops.

Data from 141 plots showed a strong positive correlation between the amount of wilt in 1938 or 1940 and the amount of wilt on the same plots when planted in tobacco 2 or 4 years later.

Wilt was usually, but not always, more severe on the low-lying areas of fields.

The similarity of the pattern of occurrence from year to year suggests that the uneven distribution was associated with permanent soil conditions rather than the random spread of inoculum by cultivation or surface water.

TOBACCO EXPERIMENT STATION, OXFORD, NORTH CAROLINA

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RHIZOCTONIA ROOT CANKER OF ALFALFA (MEDICAGO SATIVA)[1]

OLIVER F. SMITH[2]

(Accepted for publication April 1, 1943)

The Rhizoctonia root canker of alfalfa herein described was first observed by the writer at the U. S. Yuma Field Station, Bard, California, in May, 1940. An examination of alfalfa roots from many experimental plots on the station showed that a large percentage of the plants were affected, and it appeared that affected plants were being damaged considerably. It is difficult to determine how long the disease has been present in that region, but information obtained by correspondence and conversation with S. H. Hastings and E. G. Noble, Division of Irrigation Agriculture, Bureau of Plant Industry, indicates that it has been present for several years. As far as the writer is aware, no previous description of the disease has been published, nor has its cause been determined. Experiments herein reported show that the disease is caused by *Rhizoctonia solani* Kühn (*Corticium solani* (Prill. and Del.) Bourd. and Gal.).

DESCRIPTION OF THE DISEASE

The disease is characterized by dark, sunken areas, which sometimes have a brownish border (Fig. 1, B, C, D, and E). Diseased areas are usually circular, but in some cases they are oblong and extend part-way around the root. They generally occur where young roots emerge from larger ones, as evidenced by the fact that near the center of each lesion is often found the dead stub of a small root. Lesions often develop inward to the central region of the root, but the latter usually is not completely rotted off. There is very little spread of the disease up or down the root from the lesion.

The malady is seasonal in its development and closely correlated with high temperature. Lesions develop mainly in June, July, August, and September, when soil temperature, at 3 inches below the soil surface, ranges from about 21 to 35° C., whereas, there is practically no disease development during the winter months, when soil temperature, at 3 inches below the soil surface, may be as low as 5 to 10° C. Lesions that develop in summer usually heal-over during the winter, and by mid-winter or early spring there is often only a scar on the root to indicate the location of the diseased area from the previous summer season (Fig. 1, A). Under controlled conditions, the disease has developed abundantly on alfalfa roots grown at a soil temperature of 25–30° C.; whereas it has developed very little, if at all, on roots grown at a soil temperature of 16–18° C.

[1] Cooperative investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Nevada Agricultural Experiment Station.

[2] Associate Pathologist, Division of Forage Crops and Diseases.

GEOGRAPHIC DISTRIBUTION OF THE DISEASE

The disease is known to occur in southern California and southwestern Arizona, but may be present also in other areas. It has been reported[3] in the Imperial Valley of Imperial County and the Palo Verde Valley of Riverside County, California. Also it occurs in the Yuma Valley, Yuma Mesa, South Gila, North Gila, Welton-Roll, and Parker Indian Reservation Districts of Yuma County, Arizona.

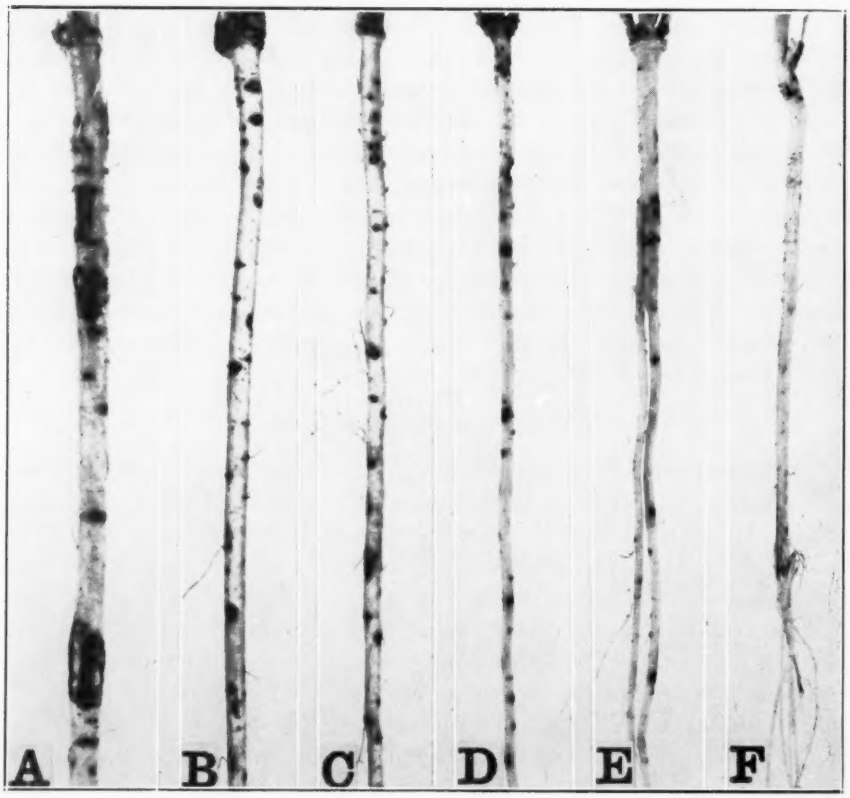

FIG. 1. Symptoms of rhizoctonia root canker of alfalfa. A. Root with scar showing diseased area largely healed over; B and C. Typical lesions on roots as a result of natural infections. D and E. Lesions on roots as a result of inoculation with *Rhizoctonia solani* at a soil temperature of 25–30° C. F. Control plant, not inoculated, but grown at a soil temperature of 25–30° C.

ECONOMIC IMPORTANCE OF THE DISEASE

The economic importance of this disease is not well defined. In regions where it is known to occur alfalfa stands are of short duration, with plant mortality being highest during the summer months, when this disease is most prevalent. It is unlikely, however, that the disease alone is respon-

[3] Unpublished data of a survey made by R. E. Beckett, Division of Irrigation Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, October, 1940.

sible for all the dying of young stands. It appears to be responsible for the death of many small roots, as well as some larger ones, and thus contributes to factors causing plant mortality. It must, therefore, be considered of real economic importance. The relative amount of resultant damage, however, awaits a more thorough knowledge of the entire disease complex in the areas where it occurs.

CAUSAL ORGANISM

The disease is caused by a fungus identified as *Rhizoctonia solani* Kühn.[4] This fungus has been isolated from many naturally developed lesions on alfalfa roots; and by inoculation experiments with this organism the disease has been reproduced and the organism reisolated.

PATHOGENICITY TESTS

Pathogenicity tests were made by growing young plants in infested soil. Plants about 3 months old were used, for at this age they bear roots large enough to test the ability of the fungus to cause root lesions. They were transplanted to galvanized-iron cans, 7 inches in diameter and 12 inches deep. In all cases transplants were allowed to grow at a soil temperature of 16–20° C., which is unfavorable for disease development, for at least 4 weeks before the soil temperature was raised to 25–30° C., which is favorable to disease development. The soil was infested with barley grain, previously sterilized, on which the fungus had grown for about 2 weeks. The grain, applied at the rate of about 20 g. (dry weight, prior to soaking and sterilization) to 7000 g. of soil, was added either by mixing with the soil just before transplanting the plants, or the transplants were allowed to grow about a month in the non-infested soil; then 5 holes, about 0.25 inch in diameter and 8 to 10 inches deep, were made in each can of soil, and the inoculum placed in the holes. Both methods gave satisfactory results.

A sandy-loam soil from Nevada was used for growing the plants, both before and after transplanting. Since experiments conducted by growing plants in this soil showed that the disease did not develop on the roots, regardless of whether or not the soil was previously heat-sterilized, non-sterilized soil was used.

The control plants were treated exactly like the inoculated ones, except that plain sterilized barley grain was added to the soil in place of barley on which the fungus was growing.

The plants were grown at a soil temperature of 25–30° C. for 2 months, then removed, and the roots were examined for disease development. In this period, many lesions developed on the roots of inoculated plants, whereas no lesions developed on the roots of control plants (Fig. 1, F). Results typical of those obtained from inoculations are given in table 1.

[4] Identified by John E. Kotila, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

Lesions produced on alfalfa roots, as a result of inoculation tests, are very similar to those that occur on roots under natural conditions at Bard, California (Fig. 1, B, C, D, and E). There can be no doubt, therefore, that *Rhizoctonia solani* is the cause of the disease here concerned.

DISCUSSION

In the experiments herein reported it has been shown that at a soil temperature of 25–30° C. *Rhizoctonia solani* causes root lesions on alfalfa. It is entirely possible, however, that under this condition there may be other organisms capable of producing similar root lesions, or may at least be effective in furthering the progress of the lesions once they are initiated.

The relation between lesion development and the death of fine roots is

TABLE 1.—*Number and per cent of alfalfa plants with root lesions following inoculation with Rhizoctonia solani*

Series	Plants inoculated	Results[1]	Per cent infection
Series 1, inoculated	25	23/25	92
" 1, control	0	0/13	0
" 2, inoculated	26	26/26	100
" 2, control	0	0/14	0

[1] Numerator = number of plants with root lesions.

not at present fully understood. Lesions often develop on large roots where fine roots have grown, but it is difficult to determine definitely whether the lesions are initiated before or after the death of the fine roots. When the disease was first observed, it appeared that some organism was killing the fine roots and then making its way from the fine roots into the larger roots to cause the lesions. However, in inoculation experiments conducted in Nevada soil, it has been observed that in some cases the lesions develop at the base of apparently healthy roots, thus indicating that, occasionally, the lesions are initiated before the roots are killed. Also, it has been observed, with many plants examined during the summer at Bard, that many of the fine roots are dead and those not dead are dark brown; yet no lesions are present on the larger roots. While it is possible that in such cases the disease has not progressed into the larger roots, it appears more likely that *Rhizoctonia solani* is not responsible for all the killing of fine roots on alfalfa in the vicinity of Bard, and that some other factor, or factors, are responsible for the death of many of these fine roots.

It has been known for several years that alfalfa stands are of short duration at Bard, plant mortality being greatest during the latter part of the summer. It seems unlikely, however, that *Rhizoctonia solani* is responsible for all the killing of stands. When plants become so deprived of fine feeder roots and root nodules during the summer, as many of the plants do at Bard, it is possible that they so thoroughly exhaust their root reserves

and become so weakened that they fall an easy prey to weak soil-inhabiting parasites, which are unable to parasitize strong healthy plants. Furthermore, it is known that *Fusarium oxysporum* var. *medicaginis* is present in this area, and it undoubtedly is a factor in the death of many plants.

SUMMARY

A disease of alfalfa roots, herein called rhizoctonia root canker, which occurs on alfalfa roots in parts of southern California and southwestern Arizona, is described and its cause attributed to *Rhizoctonia solani*.

The disease is characterized by dark, slightly sunken areas, which occur on the main tap root and large lateral roots. Lesions usually occur where young roots emerge from the larger roots. They develop mainly during June, July, August, and September, but do not ordinarily develop during the cooler part of the year. Under controlled conditions, the disease has developed abundantly on alfalfa roots grown at a soil temperature of 25–30° C., whereas, it has developed very little, if at all, on roots grown at a soil temperature of 16–18° C.

During the past several years there have occurred serious losses by alfalfa growers on the Yuma Reclamation Project and certain adjoining irrigated areas. The difficulties encountered have been chiefly in the nature of loss of plants requiring frequent reseeding. In extreme cases a high percentage of the plants die within a year from seeding. The disease herein described is believed to contribute importantly to the death of alfalfa plants in the area.

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AN ANDEAN DISEASE OF POTATO TUBERS

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(Accepted for publication April 6, 1943)

While on a trip over the Andes Mountains in Venezuela during November, 1939, the writers, accompanied by G. E. Molinet, then potato specialist of the Agricultural Experiment Station, Ministry of Agriculture and Animal Husbandry of Venezuela, found a peculiar disease of potato tubers, known locally as *buba* disease,[1] in a field and in a storage house at Mucuchíes in the State of Mérida. Specimens had been sent previously from this locality to the Experiment Station at El Valle for examination.

The affected tubers had warty swellings on the surface and were otherwise misshapen, some being cracked (Fig. 1, A). Cuts across them at various places revealed the presence of numerous brownish-black specks throughout the cortex to a depth of 1 cm. or more (Fig. 1, B). Interspersed among these and also occurring throughout the pith were other specks, light-brown or rusty, which appear to be an earlier stage of development than the black ones. No indication of the disease was observed on the roots and stalks, but the plants were mature and most of them were dead at the time. It was not determined whether all tubers of a diseased plant were affected. At least 10 per cent of those in this field showed the disease. Tubers taken to the Experiment Station became reduced within a month to a dry, brown, powdery mass containing numerous sporeballs.

The *buba* disease was found only on the native varieties, Morada and Rosada. The field in which it was found adjoined an area used by the Ministry of Agriculture for testing varieties of cereals. The tubers were being harvested and the badly affected ones were discarded and left in the field. Many of those, affected and not by this disease, bore pustules of powdery scab on the surface, while some of them harbored sclerotia of Rhizoctonia. An examination of tubers in storage disclosed the three diseases.

The disease probably has been present on potatoes of that locality for many years. The altitude of the infested field is approximately 10,000 feet. It was not found in other fields in the State of Mérida, having altitudes of 8000 to 8500 feet, nor in fields in the States of Táchira, Aragua, and Miranda at still lower elevations.

A few references to this disease have been found. Abbott (1, 2) says that it is widely distributed in the mountains of Peru where it is the most important potato disease, sometimes causing complete loss of many of the affected tubers. He ascribes the disease to *Spongospora subterranea*, the

[1] The Spanish word "*buba*" means "pustule" or "small tumor." It was not learned whether the term refers to the disease here described or to powdery scab, which was also present on the same tubers, or to both. The name appears sufficiently descriptive to be applicable to the former. The term "*La roña*" (scab) has been used as the Spanish equivalent of powdery scab.

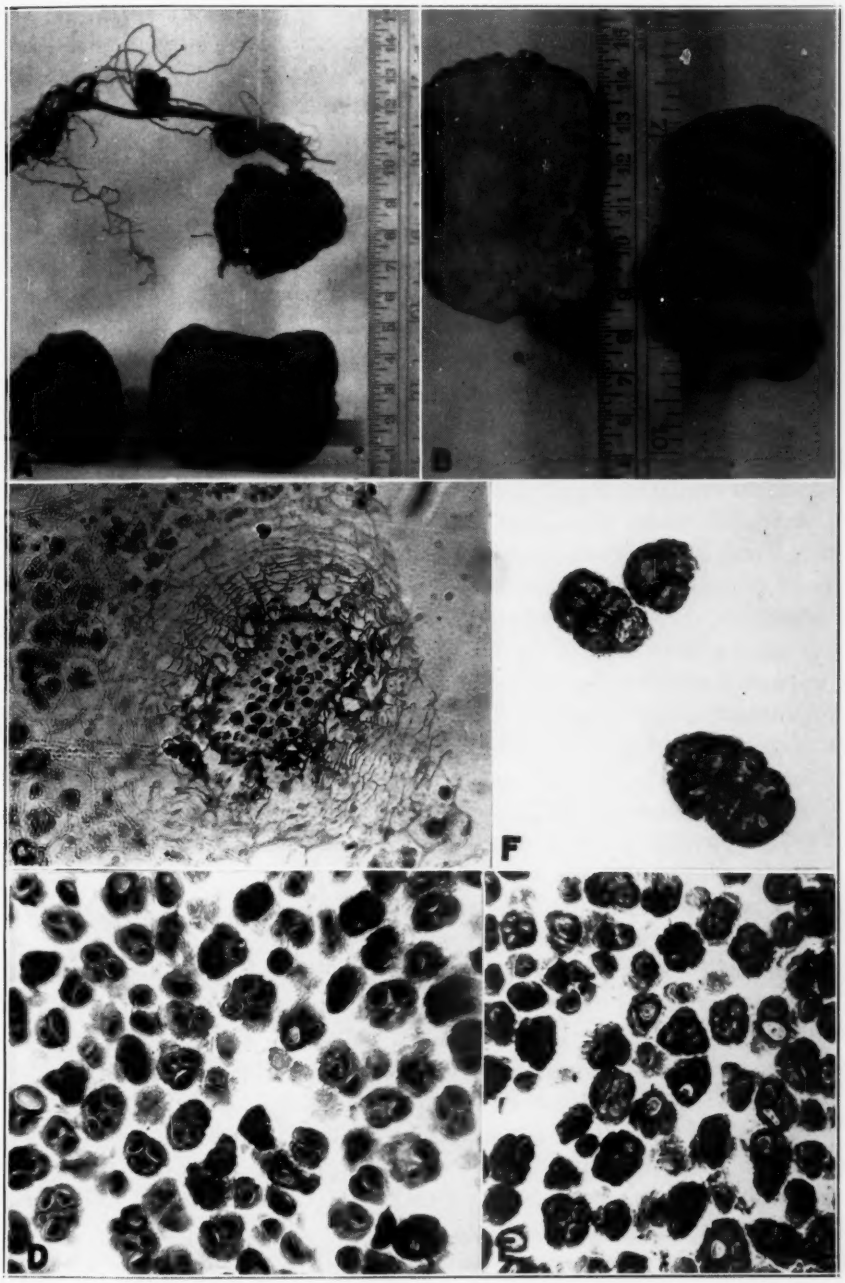

FIG. 1. *Buba* disease of potatoes. A. Malformed tubers; four small ones still attached to stem; $\frac{2}{3}$ natural size. B. A malformed tuber and a transection of it, showing numerous discolored areas in flesh; $\frac{1}{2}$ natural size. C. A section across discolored area in an early stage, showing cork cells separating the lesion from healthy cells; ×85. D. Sporeballs, from a prepared slide of a section of a discolored area. Note their thick roughened wall; ×310. E. Another section of discolored area, showing nucleated cells; ×310. F. Three sporeballs from a mount of spore dust in KOH; ×510.

pathogen of powdery scab. His descriptions and illustrations, however, include both diseases, so that one is uncertain regarding the importance and distribution of the so-called *buba* disease. Chardon and Toro (4) briefly mention a disease of potato tubers characterized by peculiar wart-like protuberances. It occurred on *papa criolla* (*Solanum sp.*), found in a field near Rincón de La Venta, State of Mérida, at an elevation about 10,824 feet. A specimen sent by them to C. L. Shear was determined as *Polysaccopsis hieronymi* (Schroet.) P. Henn., a smut producing galls on stems of *Solanum* sp. in Brazil. A note regarding this specimen occurs in a News Letter of the U. S. Bureau of Plant Quarantine, December 1, 1932 (3). From the description given there and the source of the specimen, one might conclude that the disease on the tubers collected by Chardon and Toro is the same as that collected by us. The sporeballs in our specimens, however, are not those of a *Polysaccopsis*. This has been confirmed by John A. Stevenson and C. L. Shear from specimens we sent them. Stevenson later sent the senior writer a specimen containing sporeballs of the *buba* organism from a tuber of *Solanum tuberosum* (probably *S. andigenum* Juz. et Buk.) collected at Puno, Peru, March, 1940, by J. Soukup, and a specimen of a portion of an affected tuber from Luis Rodriguez Lz. collected near Quito, Ecuador.

Specimens of affected tubers collected at Mucuchíes were taken to the experiment station at El Valle for study. Prepared slides were later made at Cornell University by Ruby R. Rice, then a graduate student, from affected tubers pickled first in 5 per cent formaldehyde solution and later transferred to a preserving solution consisting of alcohol, acetic acid, formaldehyde, and water. The specks, or lesions, consist of subglobose, oval, or irregular-shaped cavities (Fig. 1, C), ranging in size from about 500–1100 $\times$ 300–750 μ, av. 764 $\times$ 522 μ, in the darker lesions to an average of about half this size in the light-brown lesions. A few compound cavities were observed where a narrow strand of tissue separates one compartment from another. Surrounding some of the smaller cavities are 6 to 8 layers of cork cells, but only crushed cells surround the larger ones. Within the cavities are numerous bodies resembling sporeballs (Fig. 1, D), yellowish-brown, mostly subglobose to ovoid, 16–48 $\times$ 12–28 μ, av. 28.9 $\times$ 22.7 μ. These consist of 2 to 8 cells apparently permanently united, 10–16 $\times$ 7–12 μ, av. 12.5 $\times$ 9.1 μ, enclosed by a brown verrucose wall, 2–4 μ thick. A few sporeballs have only one cell. The cells are also subglobose to oval, somewhat flattened next to contiguous cells, but are variable in shape and size, even in the same ball. Each has a definite wall approximately 1 μ thick and a well-defined nucleus (Fig. 1, E). The smallest sporeballs contain the fewest cells, and the largest, the most (Fig. 1, F). The material surrounding the cluster of cells forms a kind of wall for the sporeball and extends also between some of the cells. Although it is much thinner there, it appears absent between most of them.

The cavity of the light-brown lesions contains numerous sporeballs that appear to be immature in that they are smaller than those from the darker lesions, a little lighter in color and contain fewer cells. Moreover, these

sporeballs are surrounded in the cavity by a heterogeneous material absent in the darker lesions. The early development of this organism must have occurred when the tubers were younger, since no lesions could be found with an earlier stage than that described. With increase in size of sporeballs, the cavity becomes larger, probably by destruction of the cork cells, which cannot be distinguished in the older lesions. The dry, brown, powdery mass to which affected tubers succumb contains numerous sporeballs released from the cavities.

Soil was infested at El Valle by mixing it with macerated affected tubers and placed in 12-inch pots. Healthy whole and cut Bliss Triumph tubers were planted in this soil. The pots were placed back of the laboratory in partial shade and watered frequently. The plants grew well and, when they had developed tubers 1 to 1.5 inches in diameter, the tubers were examined. A few of the tubers from some of the pots showed the typical lesions of *buba* disease. Uninfested soil produced healthy plants.

The organism, as examined, does not possess all the characters of any described fungus known to us. Before its taxonomic position can be determined with certainty, it will be necessary to germinate the spores. Repeated efforts to do this were mostly unsuccessful, but a mycelium that developed from some of the sporeballs, was cultured. Small portions of the culture were applied to the uninjured and injured surface of young Irish Cobbler tubers. This resulted in a decay of the injured tubers without *buba* disease symptoms.

The writers realize that only a start has been made in a study of the disease and its causal organism. As it is impossible at present for either writer to continue the study, it seems desirable to present at this time the information they have obtained.

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SOME MISCELLANEOUS DISEASES OF MUSHROOMS

A. M. KLIGMAN AND J. S. PENNY

(Accepted for publication April 23, 1943)

FUSARIUM SPP.

F. C. Wood in England has described a mushroom disease that he attributes to toxins produced by *Fusarium* species (principally *F. solani* (Mart.) var. *martii* (App. and Woll.) Woll. (*F. martii*) and *F. oxysporum* Schl.) in the casing soil (6,7)). The mushrooms fail to mature properly and become stunted, withered mummies with a dry, pithy, discolored interior. Wood has worked largely with the brown form of the cultivated mushroom; he has, however, found the white mushroom, also susceptible, with a resultant rapid rotting.

It was thought at first that a disease similar to this occasionally occurred in beds in the Kennett Square area. Cultures of *Fusarium solani* var. *martii* and *F. oxysporum* were isolated directly from infected pine seedlings.[1] Both species sporulated abundantly on a rye grain medium; hence, inoculated rye grains were used in infection studies. Pure-culture spawn of the brown and white varieties was packed into glass troughs following Wood's technique. The troughs were then cased with infested soil. No diseased mushrooms appeared. On many occasions mushroom beds were infested heavily in the beginning, middle, and end of the crop. Both temperature and humidity were varied. In no case was an infection detected.

In the writers' opinion, Wood's experiments do not show conclusively that *Fusarium* species cause the condition he has described. Wood emphasized the fact that the disease usually occurs under conditions of poor ventilation and a heavy crust in the casing soil. It is possible that these conditions themselves are responsible for the damage. Unfavorable casing soil is known to reduce the yield and increase the amount of "trash" or small mushrooms that do not mature. *Fusarium* species are extremely widespread in soils and may frequently be isolated from normal casing soil. There is yet no positive evidence, at least in America, that their presence in casing soil causes the injury described by Wood.

MUMMY DISEASE

Mummy disease usually appears during the last stages of the crop and generally is restricted to one or several beds. The diseased mushrooms fail to develop and remain as dry, leathery, mummies. The pileus is small, brownish, frequently tilted to one side with hard abortive gills (Fig. 1, D). The stems are bent, spindly, and shriveled. Internal tissue is tough, leathery, dry, and whitish, and with some discoloration at first in the base of the stem. Longitudinal sections frequently reveal soft discolored pits, streaks, and channels in the tissue of the cap and stem (Fig. 1, B). Later,

[1] Cultures furnished by H. Tint of the University of Penna.

the tissue becomes uniformly discolored and softer (Fig. 1, C). Greenish-gray bacterial slime often lies in the discolored areas. Globules of slime sometime occur between the gills (Fig. 1, E). *Pseudomonas fluorescens* Mig. was consistently isolated from the slime. The base of the stem is frequently swollen and covered with a velvety white overgrowth of mycelium. The disease appears suddenly on a small area and spreads rapidly in both directions until the whole bed is infected.

Shortly after this study was begun, it was learned that Tucker[2] in Missouri had already described the mummy disease without determining the

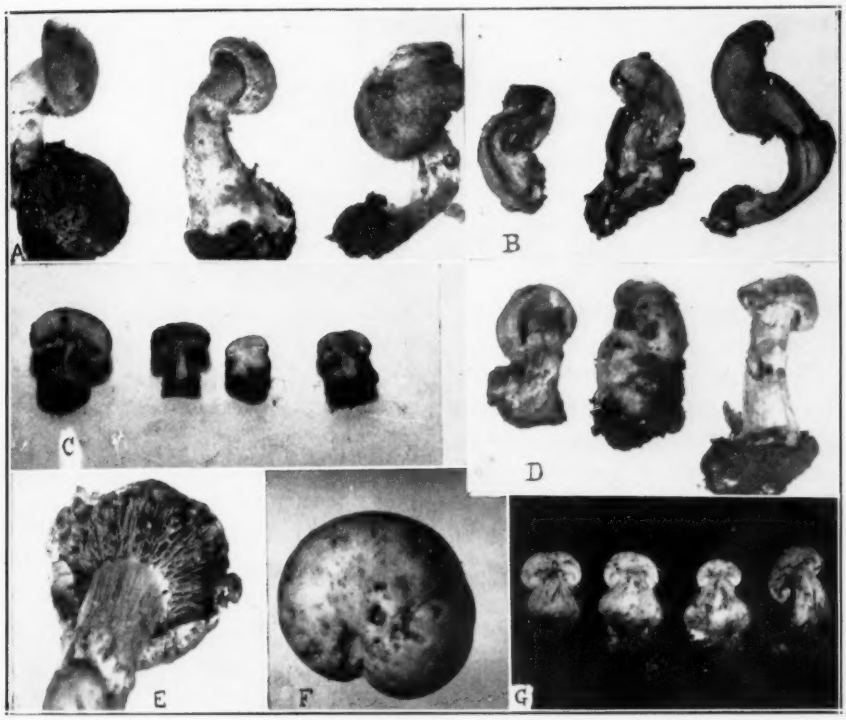

FIG. 1. A. Mummied mushrooms showing swollen base and dwarf cap. B and C. Interior of mummies showing pits and discoloration. D. Characteristic mummies. E. Bacterial slime on gills of a mummied mushroom. F. "Bacterial pit." The injury is probably caused by a mite. G. Interior of mummies collected in Missouri by J. B. Routien.

causal agent (6). He isolated a number of fungi and bacteria from infected mushrooms but was unable to show that any of these caused the disease. The writers have had similar negative results. Organisms isolated from the soil around infected mushrooms likewise produced no evidence of infection in inoculation tests.

Once the disease has appeared, its spread down the bed may be prevented by digging a trench 1 foot wide across the bed. A trench should be

[2] Personal correspondence with J. B. Routien, University of Missouri, established the identity of the Missouri disease with the one in Pennsylvania.

dug on each side about 5 feet in advance of the disease. A Formalin drench proved effective in the only 2 instances in which it was tried. The bed was allowed to dry for about 10 days after picking all mushrooms. A 2 per cent Formalin solution was then sprayed over the soil at the rate of 1 gal. per 55 sq. ft. The bed was watered again after all trace of the formaldehyde had disappeared. All mushrooms appearing in the bed thereafter were normal.

Tucker found that the disease could be spread by transferring infected soil to a healthy bed. This has been confirmed in some cases. Apparently the causal agent is transmitted through the soil; nevertheless, the disease has appeared in steam-sterilized soil. This disease differs from that described by Wood in the following complex of symptoms: conspicuously tilted cap, bent and shriveled stipe, swollen base, and pitted, discolored interior containing *Pseudomonas fluorescens*. It differs also in its tendency to spread rapidly through the mushroom bed.[3]

BACTERIAL PIT

Bacterial pit has been described by Beach (1). Small irregular pits, varying from several mm. to 1 cm., occur in the surface of the cap (Fig. 1, F). Greenish-gray masses of bacterial slime frequently lie in the pits. The internal tissue is not affected. Infrequently, a pit forms just beneath the surface.

Bacteria isolated from the slime proved to be *Pseudomonas fluorescens*. Every attempt to induce the characteristic pits by inoculating mushrooms with *P. fluorescens* failed. The suspicion arose that a small pest or insect might be responsible for the injury. Extensive examination with a hand lens on many different occasions failed to reveal pests within the pits. Infected mushrooms were then examined under a binocular microscope. Careful scrutiny revealed the presence of a small sluggish, white mite buried in the slime of some of the pits. The mites were visible with a hand lens only when outside of the pits, but became concealed as soon as they crawled into the slime. The mite belongs to the family *Tyroglyphidae* and probably is a member of the genus *Rhizoglyphus*.[4] Evidently the bacteria within the pits are secondary, living perhaps upon feces deposited by the mites. *P. fluorescens* isolated from the pits failed to grow on sterile living or dead mushroom-tissue. The cultures of *P. fluorescens* isolated from pitted mushrooms were similar to those isolated from mushrooms showing the symptoms of mummy disease. Other mites have been observed on pitted mushrooms but none has been observed directly within the pit. The pits caused by several species of *Tyroglyphus* mites do not resemble those under discussion. The former are dirtier, lack slime, and contain many *Tyroglyphus* mites (5). Certain evidence suggests that the mite in the present study is frequently

[3] Since this paper was submitted for publication, C. M. Tucker and J. B. Routien have published an exhaustive report on this disease (Mo. Agr. Exp. Stat. Bull. 358. 1942). Our much briefer work is, however, substantially in accord with theirs.

[4] The mite was first observed in the presence of C. A. Thomas of the Pennsylvania State College and was identified tentatively by him.

brought in with the casing soil. Sterilization with tear-gas or steam should be effective in eliminating them in the casing soil, although in one case sterilization with formaldehyde, which is not insecticidal, definitely eliminated them.

The term "bacterial" pit is inaccurate, if the pit actually is caused by a mite. Studies are under way to determine the efficacy of various insecticides to prevent the spread of the damage after injury has appeared on the beds.

BACTERIAL BLOTCH

Mushrooms affected with bacterial blotch develop dark, chocolate-brown spots over the cap, which may finally merge. The discoloration is entirely superficial. Paine named the pathogen *Pseudomonas tolaasi* (3). The organism has been renamed *Phytomonas tolaasi* (Paine) Bergy *et al.* on the ground that the genus *Pseudomonas* does not include pathogenic species (2). Fermentations and other biochemical reactions of *Phytomonas tolaasi* were essentially similar to those of *Pseudomonas fluorescens* isolated from pitted and mummied mushrooms. The disease is extremely rare in the Kennett Square area and has never been observed there despite extensive observations by the writers. It appears to be fairly common in the mushroom caves of Butler county, Pennsylvania. A culture was secured from an infected mushroom taken from a cave and a brief study was made on the behavior of the organism in houses in the Kennett Square area. Young and old mushrooms inoculated with suspensions always developed the characteristic symptoms. The infection was more severe under conditions of high humidity. When the casing soil or manure was inoculated with suspensions before the mushrooms had begun to appear, no infection was detected. After a group of directly inoculated mushrooms was picked the subsequent crop from the same place was disease-free. Attempts to establish the organism in the beds so that successive "breaks" would become infected failed. Evidently, there is little danger of the disease becoming important in the Kennett Square area. In caves the probable control of the disease lies in so regulating the ventilation that the surface of the mushrooms does not remain permanently wet.

Steiner has suggested the very likely possibility that the nematode, *Rhabditis lambdiensis* Maupas, is responsible for the transmission of this disease (4). This is well borne out by the fact that nematodes are common in the wet caves, where the disease is prevalent and are rather rare in the Kennett Square area, where the disease is of insignificant importance; hence, a complete control of the disease in the caves would probably demand a control of nematodes.

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A WATER SOLUBLE PROTECTANT FUNGICIDE WITH TENACITY[1]

ALBERT E. DIMOND,[2] JOHN W. HEUBERGER,[3] AND JAMES G. HORSFALL

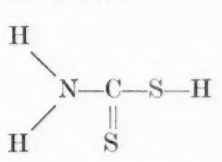
(Accepted for publication August 12, 1943)

Since protective fungicides must resist rain in order to function during infection periods, they usually have been selected on the basis of water insolubility. Water insolubility imposes the limitation that the protectants have to be composed of discrete particles. Discrete particles cannot produce a complete coverage over the surface to be protected. Inevitably, spaces are left between.

A new protectant fungicide has been discovered that is water-soluble. It goes into solution in water and forms a clear yellow liquid, which exhibits a small degree of capillary activity. The combination of the two properties of water solubility and surface activity permits the formation of an invisible film type of coverage. Thus, there are no spaces between particles, since there are no particles, and the capillary activity promotes wetting and spreading actions that assure smaller spaces between droplets than in the case where water is used as a vehicle for carrying particles. When the deposit on the foliage dries, it becomes water-insoluble, hence, resistant to removal by rain.

The compound is disodium ethylene bisdithiocarbamate, the preparation of which has been described recently by Hester.[4] The fungicidal properties of the compound were discovered in January, 1941, when it was selected in the laboratory through bio-assay from 258 organic compounds tested that month. It has been developed under the code number of **He175**. Chemically, of course, the compound is related to the salts of dithiocarbamic acid, which were originally described as fungicides by Tisdale and Williams[5] and later by Tisdale and Flenner.[6]

Dithiocarbamic acid has the formula:



[1] Approved for publication by the Director of the Connecticut Agricultural Experiment Station. Part of the research project conducted in cooperation with the Crop Protection Institute. Cost of publication of this paper was defrayed by the Crop Production Institute. The writers wish to express their thanks to Dr. W. F. Hester of Röhm & Haas Company, Inc., Philadelphia, Pa., for providing the experimental samples of disodium bisdithiocarbamate.

[2] At present assistant Professor of Botany, University of Nebraska, Lincoln.

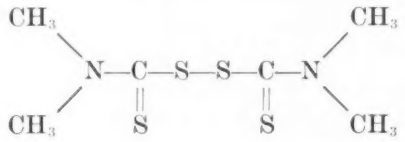
[3] At present Associate Plant Pathologist, University of Delaware, Newark.

[4] Hester, W. F. Fungicidal composition. U. S. Patent 2,317,765. 1943.

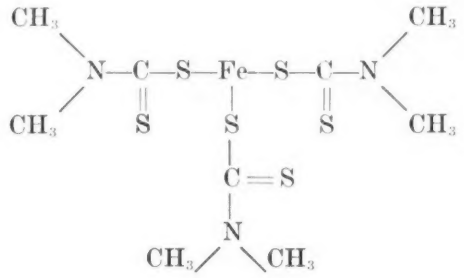
[5] Tisdale, W. H., and I. Williams. Disinfectant. U. S. Patent 1,972,961. 1934.

[6] Tisdale, W. H., and A. L. Flenner. Derivatives of dithiocarbamic acid as pesticides. Ind. and Eng. Chem. **34**: 501–502. 1942.

Two molecules can be joined through sulphur atoms by reaction of a dithiocarbamate with sulphur chloride or an oxidizing agent. By way of example, an N-dimethyl dithiocarbamate yields tetramethyl thiuram disulphide:

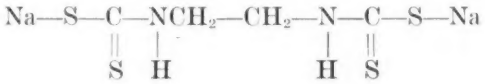


This compound is sold as a fungicide under the names of "Thiosan" and "Arasan." The ferric salt of dimethyl dithiocarbamic acid, ferric dimethyl dithiocarbamate:



is sold to the trade under the name of "Fermate."

Disodium ethylene bisdithiocarbamate:



differs from the above dithiocarbamic acid derivatives in that two molecules of dithiocarbamic acid are joined not through sulphur, but through a divalent hydrocarbon group attached to the nitrogen atom of the respective thiocarbamate radicals.

The important practical difference between the new compound and other derivatives of dithiocarbamic acid is its water solubility, its film-forming ability, and its property of becoming water-insoluble on drying. Both tetramethyl thiuram disulphide and ferric dimethyl dithiocarbamate are particulate protectants. The deposit of the latter is coal black and hence objectionable on many plants. Of course, ordinary sodium dimethyl dithiocarbamate is soluble in water like disodium ethylene bisdithiocarbamate, but sodium dimethyl dithiocarbamate is not capable of becoming insoluble on drying.

A dried deposit of the compound shows the same peculiar double-maximum toxicity curve that was described by Dimond *et al.*[7] for tetramethyl thiuramdisulphide. This peculiar situation permits the compound to have

[7] Dimond, A. E., J. G. Horsfall, J. W. Heuberger, and E. M. Stoddard. Role of the dosage-response curve in the evaluation of fungicides. Conn. Agr. Exp. Stat. Bull. 451. 1941.

three LD50 values, when one is the rule. The material, therefore, cannot be compared to another compound as usual by means of equal LD values.

Tenacity is equally difficult to assay biologically. At present, the common bio-assay for tenacity is to take a ratio of the LD50 values of a compound before and after a standard wash, according to Heuberger's procedure.[8] Since disodium ethylene disdithiocarbamate has three LD50 values, it is possible to have a higher LD50 value after washing than before, and this has happened in testing. Nevertheless, disodium ethylene bisdithiocarbamate resists weathering well in the field.

In our tests disodium ethylene bisdithiocarbamate has given promising control of *Diplocarpon rosae* and *Sphaerotheca pannosa* on roses, *Venturia inaequalis* on apple, *Cercospora apii* on celery, and *Pythium ultimum* on pea seeds. Because of the surface activity of the material, drain-off during spraying occurs sooner than for most protectants. Hence the deposit does not build up as well as that for the particulate protectants. This drain-off is particularly noticeable on easily wetted foliage, like that of potato and tomato. Probably for that reason the material has not given as satisfactory control as expected for *Alternaria solani* on tomato and *Phytophthora infestans* on potato.

The compound has shown little phytotoxicity. There has been a faint suggestion of minute light-colored spots on roses, but their effect was negligible at effective dosages.

The properties of the compound suggest several possible uses. It should prove useful to cherry and peach growers for application to near-ripe fruit, where residues are objectionable. In war time it would be of interest to gardeners who have sprayers without agitators, since the material does not settle out of suspension. It might prove interesting on onions where the foliage is difficult to wet. It should appeal to growers of ornamentals because of the absence of conspicuous residue.

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NEW HAVEN, CONN.

[8] Heuberger, J. W. A laboratory biological assay of tenacity of fungicides. Phytopath. 30: 840–847. 1940.

CONTROL OF SOIL-BORNE ORGANISMS THAT CAUSE ROTS OF GARDEN PEAS

M. F. Babb and G. W. Bohn[1]

(Accepted for publication March 16, 1943)

INTRODUCTION

In certain types of physiological investigations with garden peas (*Pisum sativum* L.) it is desirable to grow the plants in soil-jar cultures. When somewhat alkaline field soils (pH 7.2 to 7.7) at the Cheyenne (Wyoming) Horticultural Field Station are used for this purpose it is often difficult to secure good stands of plants because of seed and seedling diseases. The diseases also occur in the field at the Cheyenne Station, but seldom reach epidemic proportions. Preliminary pathogenicity work indicates that several diseases may be involved. A virulent oomycete and a pathogenic species of *Fusarium* have been isolated from diseased plants grown in the field and in nontreated field soil placed in jars in the greenhouse. These and certain other isolated bacteria and mold fungi are being studied further. That several organisms may be involved is not unexpected because many species are known to be associated with seedling and root diseases of peas. Harter, Zaumeyer, and Wade[2] listed 9 organisms causing root rots of peas and stated that others may occur. Following repeated failures to obtain good stands from pea seeds treated with the commonly recommended seed fungicides (Ceresan, Semesan, Spergon), two experiments were set up to determine the most suitable method of obtaining good stands of peas in soil-jar cultures by treating the seed or soil, or both, with various sterlizing agents.

MATERIALS AND METHODS

The tests were conducted in the Station greenhouses during the winter of 1941–1942. Temperatures ranged from 75° F. to 85° F. during the day and from 60° F. to 70° F. at night. The soil used for the treatments came from a station field where the diseases were known to occur. All of the chemical soil treatments (table 1) were applied directly to soil in 2-gal., glazed, earthenware jars provided with drainage holes. Small rocks, covered with a thin layer of sphagnum moss, were placed in the bottom of each jar to facilitate drainage. Three soil samples, designated as Libby Flat, Pole Mountain Creek, and Laramie Airport, came from areas never before cultivated and relatively remote from cultivated areas. These samples were left untreated for comparison with the treated and nontreated station soil. The seed treatments were applied by shaking the seeds with an excess of the specified dust.

[1] Cheyenne Horticultural Field Station, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

[2] Harter, L. L., W. J. Zaumeyer, and B. L. Wade. Pea diseases and their control. U. S. Dept. Agr. Farmers' Bull. 1735, 24 p. 1934.

TABLE 1.—*Effect of soil and seed treatments on number of surviving pea plants from 30 seeds sown*

Treatment rank	Seed treatments	Soil treatments	Planting delayed Series 1	Planting delayed Series 2	Average number of surviving plants from 30 seeds sown, 4 replications — Series 1	Series 2
			Days		*Number* $S_{\bar{x}}$	*Number* $S_{\bar{x}}$
1	None	40% formaldehyde solution, 1:50, 1 liter	14	14	28.50 ± 0.65	28.50 ± 0.65
2	Spergon	Sphagnum, 2″ on top of soil	No	No	....	28.00 ± 0.71
3	None	Laramie Airport virgin soil, pH 8.0	No	No	28.00 ± 0.22	....
4	None	Live steam, 6 hours	1	1	26.50 ± 1.71	27.50 ± 1.50
5	Spergon	40% formaldehyde solution, 1:50, 1 liter	14	14	....	27.00 ± 1.35
6	Spergon	Chloropicrin, 2.2 oz., 4″ deep	14	14	....	26.75 ± 1.11
7	Spergon	Live steam, 6 hours	1	1	....	26.75 ± 0.75
8	None	Sphagnum, 2″ on top of soil	No	No	25.25 ± 0.75	26.50 ± 0.65
9	None	Chloropicrin, 2.2 oz., 4″ deep	14	14	26.75 ± 0.25	24.50 ± 2.10
10	None	Libby Flat virgin soil, pH 5.5	No	No	25.50 ± 0.50	....
11	None	Pole Mountain virgin soil, pH 6.5	No	No	24.75 ± 0.85	....
12	Ceresan, New Improved	None	No	No	26.25 ± 0.75	20.50 ± 3.86
13	Semesan	None	No	No	19.50 ± 1.56	25.00 ± 1.08
14	None	Pyroligneous acid, 5%, 500 ml.	14	11	22.75 ± 1.60	19.25 ± 1.32
15	Spergon	None	No	No	17.50 ± 2.63	21.75 ± 0.85
16	None	40% formaldehyde solution, 1:6, 105 ml.	3	10	11.00 ± 1.08	27.75 ± 1.93
17	None	Formaldehyde dust,[a] 2 oz., mixed, top 4″	3	10	13.00 ± 2.97	23.75 ± 1.38
18	Ceresan, New Improved	Live steam, 6 hours	1	1	....	14.50 ± 0.65
19	Cuprocide (Red)	None	No	No	3.75 ± 2.18	20.50 ± 3.23
20	None (Check)	None (check) pH 7.2 to 7.7	No	No	3.25 ± 0.75	18.75 ± 1.80
21	Ceresan, New Improved	Sphagnum, 2″ on top of soil	No	No	....	9.75 ± 1.44
22	Ceresan, New Improved	40% formaldehyde solution, 1:50, 1 liter	14	14	....	8.75 ± 4.52
23	None	Potassium permanganate, 3%, 1 liter	No	10	0.25 ± 0.25	6.00 ± 1.58
24	Ceresan, New Improved	Chloropicrin, 2.2 oz., 4″ deep	14	14	....	2.75 ± 1.18
25	None	Sucrose, 2 oz., mixed, top 4″	14	14	....	2.50 ± 0.65
26	None	Acetic acid, 1%, 500 ml.	14	14	3.25 ± 1.70	1.25 ± 0.48
27	None	Sucrose, 1 oz., mixed, top 4″	14	14	....	2.25 ± 0.63
28	None	Sphagnum ½, soil ½, mixed	No	No	0.75 ± 0.25	2.00 ± 0.58
29	None	Sucrose, 4 oz., mixed, top 4″	14	14	1.00 ± 0.71	....
30	None	Sucrose, 8 oz., mixed, top 4″	14	14	0.00	....

[a] Formaldehyde dust made from formaldehyde (40%) 1 lb., infusorial earth 6 lbs.

Four jars were used for each treatment, and the treatments were randomized on a greenhouse bench. Thirty peas of the Laxtonian variety were sown in each jar. No record was taken of the time of emergence of the plants. However, emergence was rather uniform in the checks and beneficial treatments, but was delayed in certain of the injurious treatments. The plants were allowed to grow until they were in bloom, when counts were made of those surviving. Two series of tests were made. In the first, either the soil or the seed was treated; in the second series the most successful soil and seed treatments of the first were used alone and in combination. The nature of the treatments together with the results of the two series of tests are summarized in table 1.

DISCUSSION

The treatments are listed in table 1 in the order of their effectiveness in controlling the diseases as shown by plant survival. The order of listing the treatments was determined in some cases by only one test; but in those instances where a treatment was repeated the order of listing was determined by averaging the results of two tests.

The average plant survival for the checks in the two tests was low or ranked in 20th place among the 30 treatments. There was, however, considerable difference in plant survival between the checks in the two tests. As all seed came from the same package but the soil from different parts of the field, this variation in survival suggests that the diseases were soil-borne and that there was a difference in the kind or abundance of parasites in various parts of the field. There was also a considerable lapse in time between the first and second tests during which the soil was exposed to the action of the sun and drying winds. It is possible that these factors also had some effect on soil infection.

Treatment of the soil in 2-gallon jars with 1 liter of a 1/50 dilution of 40 per cent formaldehyde (1),[3] with live steam for 6 hours (4), or with 2.2 oz. of chloropicrin (9) yielded good stands of peas. Treatment of the seed with New Improved Ceresan (12), Semesan (13), Spergon (15), or Red Cuprocide (19) gave less satisfactory control. Spergon did not affect the efficiencies of the soil treatments (5, 6, and 7). New Improved Ceresan apparently caused injury when used in combination with these soil treatments (18, 22, 24).

The good stands of plants obtained from nontreated seeds in steamed soil (4), in nontreated, virgin soils from Laramie Airport (3), Libby Flat (10), and Pole Mountain (11), and in sphagnum moss (8) in comparison with the poor stands secured in soil from the station experimental field (20) indicate that the diseases were largely soil-borne. There was little indication of any seed-borne diseases. This conclusion is further substantiated by the very poor stands obtained in the soil and sphagnum moss mixture (28), and from the fact that the seed treatments alone (12, 13, 15, 19) failed to control the disease as well as did the effective soil treatments (1, 4, 9).

[3] Figures in parentheses refer to treatment rank given in table 1.

A NONINFECTIOUS HERITABLE LEAF-SPOT AND SHOT-HOLE DISEASE OF THE BEATY PLUM[1]

C. O. Smith and L. C. Cochran[2]

(Accepted for publication March 9, 1943)

Several nontransmissible, tissue-perpetuated, virus-like abnormalities have been observed on Prunus plants. Kinman[3] described a trouble on sweet cherry, in California, locally known as "crinkle leaf" and characterized by variegation and malformation of the leaves and a sparse set of small, misshapen fruits. Nursery stock grown from buds from affected trees perpetuated the trouble, and affected orchard trees top-worked with cions from normal trees developed normal tops. Blodgett[4] described a leaf spot of the Italian prune associated with defoliation and severe decrease in vigor. This disorder was similar to that of the cherry in that it was perpetuated in the growth from diseased cions, but was not transmitted to the healthy stocks.

A disease, apparently of the same class as the two listed above, has been under observation for a number of years in seedlings of the Beaty plum. These seedlings were in a planting at the University of California Citrus Experiment Station, Riverside, and were grown from pits from a tree that was purchased from a Texas Nursery Company under the variety name of El Paso. This variety is listed by Wight[5] as a synonym of the Beaty plum, a hybrid between *Prunus angustifolia varians* and *P. munsoniana*. Certain of these seedlings have shown spots and shot holes in their leaves (Fig. 1, A) annually, while others have remained free.

The affected seedlings varied greatly in symptom expression, some being severely and others only mildly affected. Early spring growth on the affected trees appeared to be normal. As the season progressed, the older leaves at the base of the shoots developed translucent flecks that rapidly enlarged. The centers of these spots turned brown (Fig. 1, B), and in the majority of cases, the brown areas separated from the rest of the leaf at a definite line of cleavage and dropped out. The resultant shot-hole effect developed progressively toward the apex of the shoots, and, by midsummer, when growth had become slower, the foliage appeared riddled with holes (Fig. 1, A). The spots and holes ranged from pin-point size to a diameter

[1] Paper No. 492, University of California Citrus Experiment Station, Riverside, California.

[2] University of California Citrus Experiment Station, Riverside, California, and the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

[3] Kinman, C. F. A study of some unproductive cherry trees in California. Jour. Agr. Res. [U. S.] **41**: 327–335. 1930.

[4] Blodgett, Earle C. A leaf spot of Italian prune perpetuated in budded stock. Phytopath. **30**: 347–348. 1940.

[5] Wight, W. F. The varieties of plums derived from native American species. U. S. Dept. Agr. Bull. 172: 1–44. 1915.

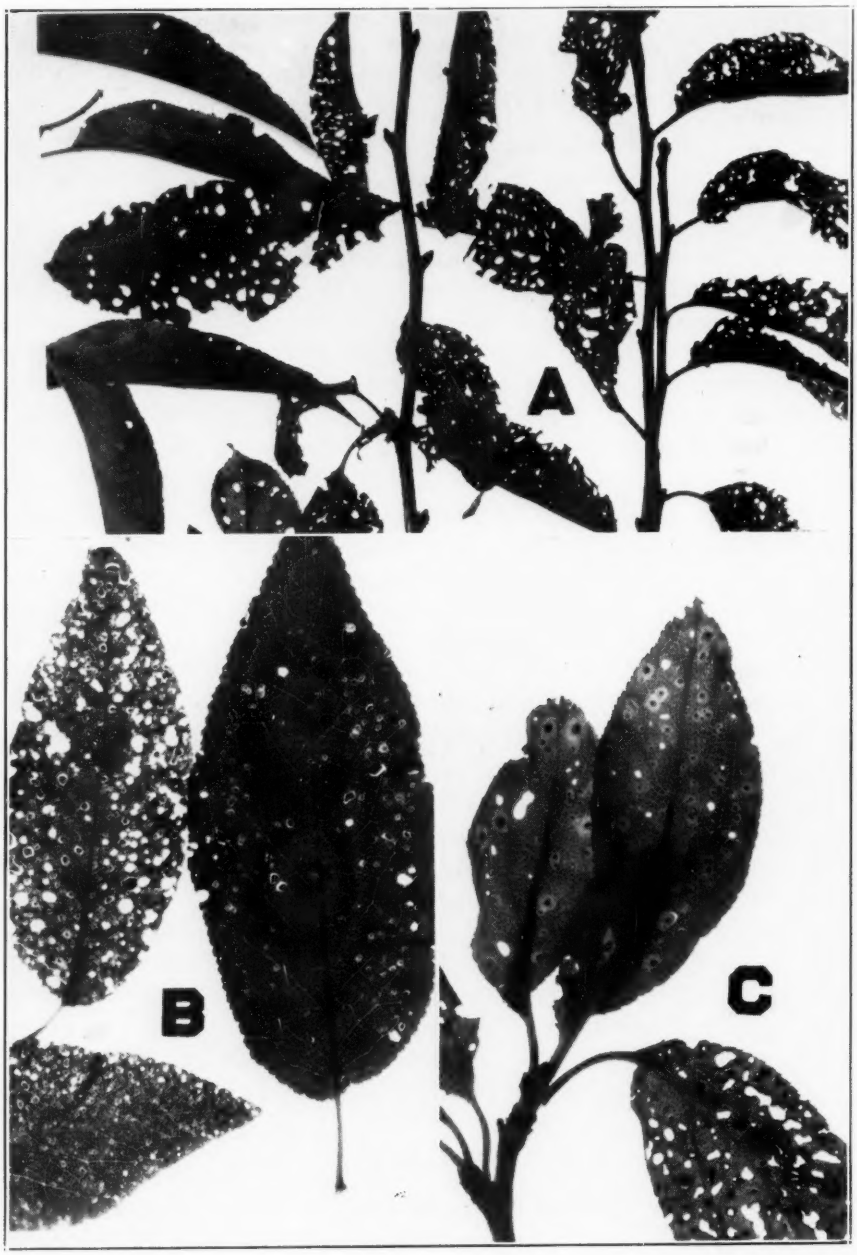

FIG. 1. Leaves of Beaty plum seedling, showing leaf spots and shot holes. A. Shot holes in an advanced stage. B. Shot holes in leaves on shoots from buds that had been taken from a diseased tree and budded into peach stock. C. Shot holes showing the zone about a dark center.

of 1 to 2 mm., the size being inversely proportional to the number on the affected leaf; they were usually circular, but were often of irregular shape where they coalesced or occurred near or in the angles of veins.

The shot-hole condition described above is not macroscopically distinct from that caused by the fungus *Coryneum beijerinckii* Oud., but it develops at a season unfavorable to the fungus. Cultures from the spots yielded no organisms known to cause such injury. This condition is distinguished from a transmissible ring-spot and shot-hole condition described by Cochran and Hutchins[6] on peach, as will be shown from the evidence presented later in this paper.

An attempt was made to inoculate the following species of *Prunus* with buds taken from a severely shot-hole-affected seedling of Beaty plum: *Prunus armeniaca* L., *P. angustifolia* Marsh, *P. communis* Fritsch, *P. cerasifera* Ehrh., *P. hortulana* Bailey, *P. mexicana* Wats., *P. munsoniana* Wight and Hedr., *P. mume* Sieb. and Zucc., *P. orthosepala* Koehne, *P. umbellata* Ell., *P. persica* Sieb. and Zucc., as well as healthy seedlings of Beaty plum. In no case did any of the stocks show symptoms indicating transmission. The initial leaves on shoots from the inoculation buds at first appeared healthy, but they later developed typical spots and shot holes (Fig. 1, C) characteristic of the affected mother tree.

Cions taken from the original parent Beaty plum, which had shown no symptoms, were top-worked onto the seedlings showing the shot-hole condition. None of the growth from the cions showed any shot holes, whereas the leaves of these seedling understocks were riddled with holes.

The nontransmissibility of the disorder, the absence of shot-hole symptoms on the parent tree and on some of the seedlings, and the variation in intensity of symptoms on the affected seedlings suggest that the shot-hole condition is a genetical abnormality that segregates among the progeny. The parent tree, as previously stated, was known to be a hybrid. While the symptom characters of the disease simulate those caused by some viruses, the nontransmissible nature of the trouble precludes its inclusion in the class of known virus diseases.

[6] Cochran, L. C., and Lee M. Hutchins. A severe ring-spot virosis on peach. (Abstract) Phytopath. 31: 860. 1941.

SEVERE POTATO LATE-BLIGHT INFECTION IN SEBAGO TUBERS[1]

G. H. Rieman and John S. McFarlane

(Accepted for publication April 15, 1943)

The new Sebago potato variety has proved to be moderately resistant to late blight caused by *Phytophthora infestans* (Mont.) de Bary. In repeated tests for 10 years, very few Sebago tubers developed late-blight rot under natural field conditions where susceptible Green Mountain potatoes developed from 20 to 70 per cent decay.[2] In contrast with these findings, exceptionally severe tuber decay caused by *P. infestans* was observed in Sebago potatoes grown in a series of field trials in Central Wisconsin in 1941.

Northern-grown foundation seed stocks of Sebago and Russet Rural varieties were planted side by side in rows through the center of commercial Russet Rural fields on 19 representative sandy soil farms in Central Wisconsin in June, 1941. Most of the fields were neither sprayed nor dusted for blight control, and late-blight infection was first observed on Russet Rural vines in mid-August. On September 1 a general late-blight epidemic was in progress, which continued with increasing severity during this month. The vines of the Sebago variety gradually became infected, but at a much slower rate than did the Russet Rural vines. No frosts had occurred up to the first week in October when the paired rows of Sebago and Russet Rural potatoes were harvested. The vines were still partly green at harvest time at most of the locations. The paired rows of the two varieties were dug by hand during a period of frequent showers. Very little tuber decay was observed in the field. Yield records were taken on five 25-hill replicates in each row and a composite sample of U. S. No. 1 size tubers was set aside for further study. These samples were handled in bushel baskets and stored in a commercial potato warehouse for a period of two months.

Many of the tubers of both varieties became badly decayed and covered with typical mycelium mats and fruiting bodies of *Phytophthora infestans* during the storage period. The stored tubers were washed and carefully examined for late-blight tuber rot. The weights of healthy and of infected tubers were taken for each sample. It can be noted readily from the data presented in table 1 that the Sebago tubers showed more rot than did the Russet Rurals. This is most unusual in the light of numerous convincing reports of high late-blight tuber-rot resistance for the Sebago variety.[3] Furthermore, it was found in cutting the potatoes in the various samples that the extent of decay was much greater in the Sebago tubers than in the Russet Rurals (Fig. 1).

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station as Paper No. 314 from the Department of Genetics.

[2] Bonde, Reiner, F. J. Stevenson, and C. F. Clark. Resistance of certain potato varieties and seedling progenies to late blight in the tubers. Phytopath. 30: 733–748. 1940.

[3] Wisc. Agr. Expt. Stat. Ann. Rpt. Bull. 443: 6. 1939.

TABLE 1.—*Late blight tuber rot occurring in 19 paired trials of Sebago and Russet Rural grown in Central Wisconsin in 1941*

Grower	Sebago			Russet Rural		
	Healthy tubers	Diseased tubers	Diseased	Healthy tubers	Diseased tubers	Diseased
	lb.	*lb.*	*Per cent*	*lb.*	*lb.*	*Per cent*
1	20	30	60	23	28	55
2	48	1	2	48	0	0
3	53	1	2	48	4	8
4	50	2	4	49	3	6
5	36	10	22	47	4	8
6	5	46	90	25	28	53
7	17	35	67	35	13	27
8	52	3	5	54	1	2
9	20	31	61	23	28	55
10	41	9	18	52	2	4
11	10	42	81	11	39	78
12	28	25	47	35	17	33
13	16	34	68	48	4	8
14	19	28	60	18	32	64
15	43	10	19	48	5	9
16	7	44	86	9	43	83
17	42	7	14	46	5	10
18	38	14	27	31	20	39
19	50	0	0	44	5	10
Total	595	372	38	694	281	29

The amount of tuber decay found in the susceptible Russet Rurals was not unusual for the season and agreed with grower experiences in the test area. The question arises as to the cause of the high late-blight tuber-rot infection observed in the Sebago variety in this series of field trials. Varia-

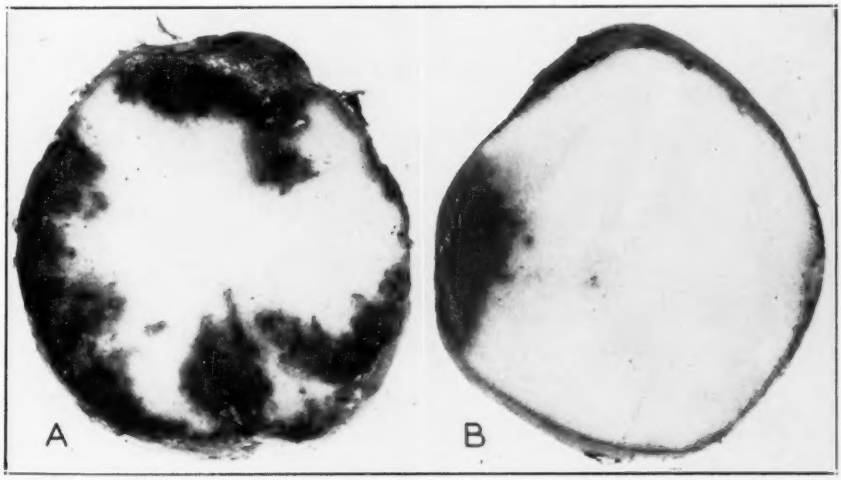

FIG. 1. Typical tuber decay caused by *Phytophthora infestans* in two potato varieties grown side by side in the field and stored for two months under identical conditions. A. Cross section of Sebago tuber showing severe dry rot decay. B. Cross section of Russet Rural tuber showing an average amount of dry rot decay in a standard susceptible variety.

tions in field-plot technique and storage are probably unimportant in dealing with this problem, since the experiment was so conducted as to give each variety identical treatment in the paired trials. The Sebago late-blight susceptibility to tuber rot noted in these tests could have developed in 3 different ways. 1. Tuber infection could have taken place early when the tubers were young and susceptible. However, had this been the case, considerable tuber decay should have been evident at harvest. 2. The Sebago vines were in much better condition than those of the Russet Rurals throughout the epidemic. Under these conditions the Sebago tubers may have been more immature than the Russet Rural tubers and, therefore, more subject to bruising during the harvesting operations. Fresh, actively sporulating growth of the pathogen was present on the vines in most of the fields, and there was ample opportunity for tuber-wound inoculation. 3. A considerable portion of the growing season was exceptionally favorable for the disease. During this extended period more virulent strains of the pathogen may have developed on the partly resistant Sebago host.[4] This would account for both the high percentage of tuber infection and the greater extent of tuber decay found in the Sebago variety.

WISCONSIN AGRICULTURAL EXPERIMENT STATION,
MADISON, WISCONSIN.

[4] Mills, W. R. Adaptive parasitism of Phytophthora infestans. Phytopath. 30: 17. 1940.

Reddick, D., and W. Mills. Building up virulence in Phytophthora infestans. Amer. Potato Jour. 15: 29–34. 1938.

ANNOUNCEMENT

Dissolution of the Tropical Plant Research Foundation.—At a meeting on April 21, 1943, of the Board of Trustees of the Tropical Plant Research Foundation in Washington, D. C., decision was reached to dissolve the Foundation and assign its resources to other organizations more actively engaged in tropical research. The large library, including the life-time collections of the late William Allen Orton, was presented to the Inter-American Institute of Agricultural Sciences at Turrialba, Costa Rica, and dedicated as a memorial to Dr. Orton. Residual funds were given to the National Research Council. It is contemplated that these, together with proceeds from the sale of certain publications given to the Pan American Union, may be used in support of Latin American educational and research programs.

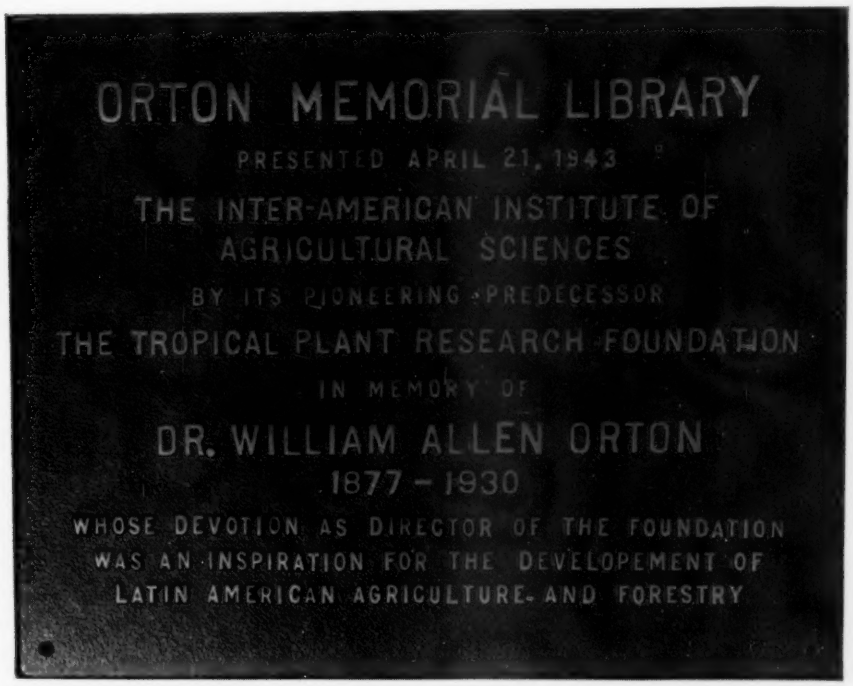
ORTON MEMORIAL LIBRARY
PRESENTED APRIL 21, 1943
THE INTER-AMERICAN INSTITUTE OF
AGRICULTURAL SCIENCES
BY ITS PIONEERING PREDECESSOR
THE TROPICAL PLANT RESEARCH FOUNDATION
IN MEMORY OF
DR. WILLIAM ALLEN ORTON
1877 - 1930
WHOSE DEVOTION AS DIRECTOR OF THE FOUNDATION
WAS AN INSPIRATION FOR THE DEVELOPEMENT OF
LATIN AMERICAN AGRICULTURE AND FORESTRY

FIG. 1. Bronze tablet in memory of Dr. William Allen Orton presented with his library to the Inter-American Institute of Agricultural Sciences, Turrialba, Costa Rica, by the trustees of the Tropical Plant Research Foundation at their final meeting disbanding the Foundation.

The Foundation was dependent upon grants for specific projects from interested business or official agencies, but has been inactive for a number of years. Its extensive researches during the 1920's for the Cuban sugar industry and its agricultural and forestry surveys in several countries have contributed materially toward sound organization of the present inter-

American agricultural research programs. The remaining trustees of the Foundation were L. R. Jones, president of the board, William Crocker, acting director and general manager, R. A. Harper, Herbert Osborn, and S. C. Prescott.

The vision and pioneering activities of Dr. Orton in the advancement of agricultural investigations for improvement of inter-American economic relations were reviewed at the meeting and subsequently expressed on a bronze tablet accompanying his library to Turrialba. As one of the organizers, a charter member and former president of the American Phytopathological Society[1] this tablet presents a fine tribute and enduring reminder of his contributions in a broader field during his later years. Since so few will visit Turrialba in the near future at least, a photograph (Fig. 1) of the memorial tablet is reproduced with this notice.—R. D. RANDS, Rubber Plant Investigations, Plant Industry Station, U. S. Department of Agriculture, Beltsville, Maryland.

[1] Jones, L. R. William Allen Orton, 1877–1930. Phytopath. 21: 1–11. 1931.